Canada

Fisheries and Oceans | Pêches et Océans

Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

Fisheries and Oceans | Pêches et Océans

Information and Publications Branch | Direction de l'information et des publications

Ottawa K1A 0E6

The *Canadian Journal of Fisheries and Aquatic Sciences* has been published continuously since 1901, previously as *Contributions to Canadian Biology* 1901–25, *Contributions to Canadian Biology and Fisheries* 1926–34, *Journal of the Biological Board of Canada* 1934–37, and *Journal of the Fisheries Research Board of Canada* 1938–79.

Editorial policy

The *Journal* publishes original research articles and notes, critical reviews, Perspectives (essays of opinion or hypothesis), comments, and book reviews. Papers may concern cells, organisms, populations, ecosystems, or processes that affect aquatic production systems, and they should lead to identifiable conclusions or synthesis, which variously may amplify, modify, question, or redirect accumulated knowledge embodied in contemporary perceptions of a particular state of fisheries and aquatic sciences. They should demonstrate clearly a contribution to knowledge beyond the confirmatory state. Originality should relate to more than the particular (a certain year, place, taxon, or chemical compound) such that existing understanding is reformulated or extended.

It would assist the Editors if prospective authors identified briefly by covering letter (a) aspects of their papers that meet the foregoing objectives, (b) potential referees, and (c) other manuscripts contemplated or in press containing the same or similar information.

Submissions in English or French are acceptable. The information must be original, that is, not copyrighted, published, or submitted elsewhere except in abstract form or unless by written consent of the Editor. The *Journal* accepts no responsibility for statements made by contributors. The use of proprietary names does not imply endorsement of the product or company.

A charge is made for manuscripts published. When submitting a manuscript, an author must state that the charge will be accepted.

Guides

Guides for authors and typists appear in the first issue of each volume and are available free from the *Journal*.

Subscriptions

The *Journal* is issued monthly to form annual volumes of twelve numbers and Supplements. Orders and inquiries regarding subscriptions must be sent directly to Supply and Services Canada, Ottawa, Ontario, Canada K1A 0S9. Prepayment is required in Canadian funds to the order of "The Receiver General for Canada."

Microfilm

Issues from 1934 through the current volume can be purchased on 16- or 35-mm microfilm. Photocopies of individual articles or issues can be purchased from Xerox University Microfilms, 300 North Zeeb Road, Ann Arbor, Michigan 48106, USA.

Design: Ludvic Saleh & Associates, Ottawa

Conception graphique: Ludvic Saleh & Associates, Ottawa

Publié sans interruption depuis 1901, le *Journal canadien des sciences halieutiques et aquatiques* a paru sous plusieurs titres : *Contributions to Canadian Biology* 1901–25, *Contributions to Canadian Biology and Fisheries* 1926–34, *Journal of the Biological Board of Canada* 1934–37 et *Journal de l'office des recherches sur les pêcheries du Canada* 1938–79.

Politique de rédaction

Le *Journal* publie des articles et des notes fondés sur une recherche originale, des critiques, des essais portant sur une opinion ou une hypothèse (Perspectives), des commentaires et des comptes rendus de livres. Les textes peuvent avoir trait aux cellules, aux organismes, aux populations, aux écosystèmes ou aux processus qui influencent les systèmes de production aquatique. Ils doivent aboutir à des conclusions ou synthèses précises qui, de diverses façons, peuvent accroître, modifier, remettre en question ou réorienter le bagage actuel des connaissances et perceptions dans une discipline donnée des sciences aquatiques. Ils doivent clairement démontrer qu'ils contribuent aux connaissances en faisant plus que corroborer des faits. L'originalité doit dépasser le caractère particulier (une année, un endroit, un taxon ou un composé chimique donné) et tenir à une épuration ou à une reformulation des connaissances actuelles.

Les auteurs éventuels aideraient les rédacteurs s'ils identifiaient brièvement, dans une lettre d'accompagnement (a) les aspects de leurs textes qui répondent particulièrement aux objectifs indiqués ci-dessus, (b) des arbitres possibles et (c) d'autres manuscrits envisagés ou sous presse, dont la teneur est identique ou se rapproche de celui qui est soumis.

Les contributions peuvent être en anglais ou en français. Elles doivent être originales, c.-à-d. qu'elles ne doivent pas avoir fait l'objet d'un copyright, avoir été publiées ou soumises ailleurs, sauf sous forme abrégée ou avec le consentement du rédacteur. Le *Journal* décline toute responsabilité quant aux énoncés des contributeurs. L'utilisation des noms de marques de commerce ne signifie aucunement une sanction du produit en question ou de la compagnie qui le fabrique.

Les manuscrits acceptés sont sujets à des frais de publication. Quant un auteur présente un texte, il doit préciser que ces frais seront acceptés. On trouvera dans une récente livraison du *Journal* les taux par page en vigueur.

Guides

Le guide des auteurs et celui des secrétaires d'auteurs paraissent dans la première livraison de chaque volume et sont disponibles gratuitement du *Journal*.

Abonnement

Le *Journal* paraît tous les mois, formant ainsi un volume de douze numéros et Supplements par année. On est prié d'adresser directement commandes et demande de renseignements relatifs à l'abonnement à Approvisionnements et Services Canada, Ottawa (Ontario), Canada K1A 0S9. Les paiements doivent être faits à l'avance en devises canadiennes, à l'ordre du « Receveur général du Canada ».

Microfilms

On peut acheter sur microfilms de 16 ou 35 mm tous les numéros publiés depuis 1934. Des photocopies d'articles ou de numéros individuels sont aussi en vente auprès de Xerox University Microfilms, 300 North Zeeb Road, Ann Arbor, Michigan 48106, É.-U.

Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

Volume 43, No. 10, October 1986 **Volume 43, nº 10, octobre 1986**

Information and Publications Branch / Direction de l'information et des publications

J. Watson, Ph.D.	*Director and Editor-in-Chief / Directeur et rédacteur en chef*
Johanna M. Reinhart, M.Sc.	*Deputy Director and Editor / Sous-directrice et rédactrice*
D. G. Cook, Ph.D. / Lorraine C. Smith, Ph.D.	*Assistant Editors / Rédacteurs adjoints*
J. Camp / G. J. Neville / P. M. Burke / M. L. Gilbert	*Editorial and Publishing Services / Services de rédaction et d'edition*

Editorial Board / Conseil de rédaction

L. Legendre	*Université Laval*	(1983–1986)
M. Sinclair	*Department of Fisheries and Oceans, Halifax*	(1983–1986)
C. J. Walters	*University of British Columbia*	(1983–1986)
C. M. Wood	*McMaster University*	(1983–1986)
P. J. Dillon	*Ontario Ministry of the Environment*	(1984–1987)
M. C. Healey	*Department of Fisheries and Oceans, Nanaimo*	(1984–1987)
J. J. Magnuson	*University of Wisconsin*	(1984–1987)
J. Sprague	*University of Guelph*	(1984–1987)
L. M. Dickie	*Department of Fisheries and Oceans, Dartmouth*	(1985–1988)
J. J. Dodson	*Université Laval*	(1985–1988)
S. J. Smith	*Department of Fisheries and Oceans, Dartmouth*	(1985–1988)
A. P. Zimmerman	*University of Toronto*	(1985–1988)

Editorial Office:
Department of Fisheries and Oceans
Communications Directorate
Information and Publications Branch
Centennial Towers
200 Kent Street
Ottawa, Canada K1A 0E6

Bureau de rédaction :
Ministère des Pêches et des Océans
Direction générale des communications
Direction de l'information et des publications
Les Tours Centennial
200, rue Kent
Ottawa, Canada K1A 0E6

The *Journal* is abstracted or indexed in: / Le *Journal* est résumé ou signalé dans :

Aquatic Sciences and Fisheries Abstracts, Biological Abstracts, Chemical Abstracts, Current Awareness in Biological Sciences, Current Contents, FAO Freshwater and Aquaculture Contents Tables, FAO Marine Science Contents Tables, and Science Citation Index

Growth and Genetic Variation of Atlantic Salmon (*Salmo salar*) from Different Sections of the River Alta, North Norway

Tor G. Heggberget and Roar A. Lund

Directorate for Nature Management, Tungasletta 2, N-7000 Trondheim, Norway

and Nils Ryman and Gunnar Ståhl

Department of Genetics, University of Stockholm, S-10691 Stockholm, Sweden

Heggberget, T. G., R. A. Lund, N. Ryman, and G. Ståhl. 1986. Growth and genetic variation of Atlantic salmon (*Salmo salar*) from different sections of the River Alta, North Norway. Can. J. Fish. Aquat. Sci. 43: 1828–1835.

Growth of young Atlantic salmon (*Salmo salar*) from three different sections of the River Alta was correlated with estimated growth differences among adult salmon caught in corresponding sections of the river. Young salmon grew most quickly each of the three years investigated in the upper section of the river; further downriver, presmolts had a significantly lower growth rate. Growth calculations based on scale samples from adults indicated corresponding river growth patterns from the three sections. Salmon caught in the upper section of the river had significantly lower smolt age (mean 3.92 yr) and better presmolt growth than salmon caught further downriver (mean smolt age in the middle and lower sections was 4.35 and 4.19 yr, respectively). Correlations between growth differences in young and adult salmon suggest that presmolts that have lived their first years in the upper section of the river apparently return there after having been at sea. Genetic analyses of presmolts indicate that local populations exist. Allele frequency differences at three electrophoretically detectable protein loci give independent support for the existence of genetically differentiated local populations within the River Alta.

La croissance de jeunes saumons de l'Atlantique (*Salmo salar*) de trois sections distinctes de la rivière Alta a été mise en corrélation avec les écarts de croissance estimés notés chez les saumons adultes capturés dans ces mêmes sections. Au cours des trois années de l'étude, les jeunes saumons présentant la croissance la plus rapide se trouvaient dans la section la plus en amont; plus en aval, les saumons au stade de pré-tacon présentaient un taux de croissance significativement moins élevé. Les taux de croissance calculés à partir d'écailles prélevées chez les adultes indiquaient que les allures de croissance étaient similaires dans les trois sections de la rivière. L'âge au stade de tacon était significativement inférieur (3,92 a en moyenne) et la croissance des pré-tacons était supérieure dans la section d'amont comparativement aux sections plus en aval (l'âge moyen des tacons dans la section moyenne et la section d'aval était respectivement de 4,35 et de 4,19 a). Les corrélations entre les écarts de croissance des jeunes et des adultes portent à croire que les saumons de stade pré-tacon qui avaient passé les premières années de leur vie dans la section d'amont y retournaient après avoir vécu en mer. L'analyse génétique des pré-tacons a montré l'existence de populations locales. Des écarts de fréquences d'allèles à trois locus de protéines décelables par électrophorèse appuient de façon indépendante l'existence de populations locales génétiquement différenciées dans la rivière Alta.

Received March 28, 1985
Accepted May 30, 1986
(J8173)

Reçu le 28 mars 1985
Accepté le 30 mai 1986

Several reports illustrate that individual rivers have their own Atlantic salmon (*Salmo salar*) populations. Adult salmon, after living a period in the sea, normally return to their natal river (Carlin 1968; Harden Jones 1968; Rosseland 1971). More recent studies document that reproductive units with morphological and genetic differences exist in Scandinavia and other places in the world (Clutter and Whitesel 1956; Møller 1970; Ryman and Ståhl 1981; Thorpe and Mitchell 1981). Genetic divergence of Atlantic salmon from different rivers as well as different locations within a river was reported by Ryman and Ståhl (1981), Ståhl (1981, 1983), Ryman (1983), and Ståhl et al. (1983).

There is little information on whether Atlantic salmon that are reared in one part of a river return to the same stretch of river to spawn. During recent years, a comprehensive study has taken place on the River Alta in North Norway to analyse growth patterns among presmolt Atlantic salmon from different stretches of the river. Scale samples of adult salmon from corresponding stretches of the river have also been collected. If salmon reared in one section of the river return to that section as adults, possible differences in growth patterns of presmolt salmon from different stretches would be identical to growth differences of adult salmon captured in corresponding parts of that river.

This study is an analysis of growth patterns among presmolt and adult salmon from different stretches of the River Alta to test the hypothesis that salmon originating from a specific stretch of a river return to that same stretch to spawn. This hypothesis was tested by examining the joint genetic and growth variation within three different parts of the River Alta, as indicated by electrophoretic analysis.

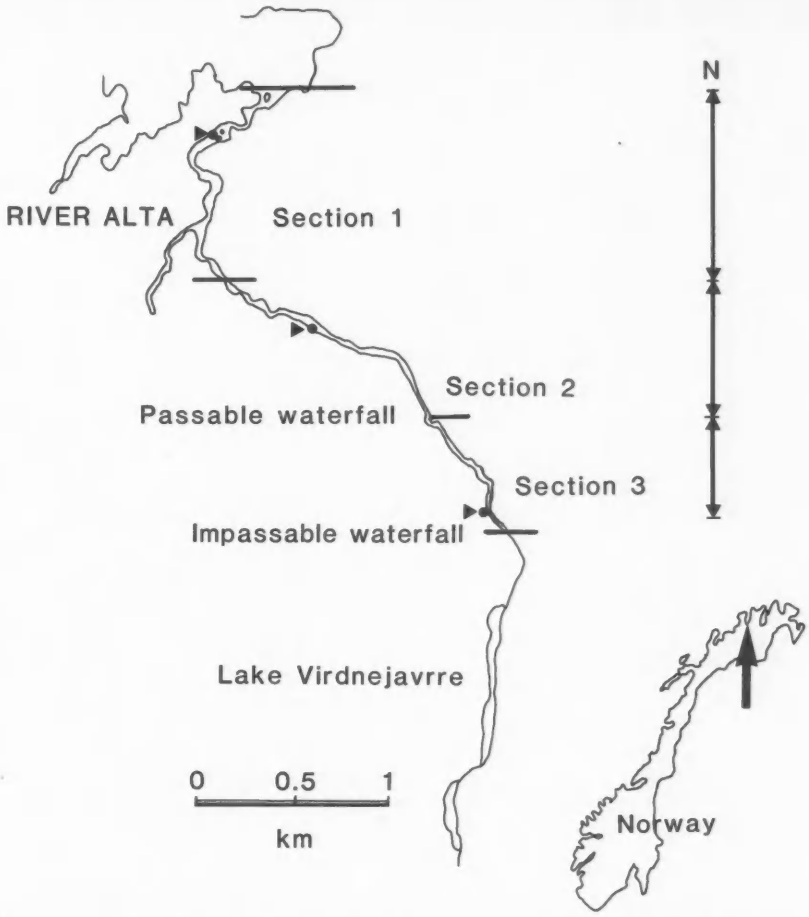


FIG. 1. The River Alta showing the three different sections of the river where the growth of salmon was analysed (▲ = sampling stations for presmolt salmon).

Study Area

The River Alta has a catchment area of 7400 km^2, and salmon can migrate approximately 46 km upstream from its outlet to the sea (Berg 1964). Atlantic salmon is the dominant fish in the Alta. Mean annual catch of Atlantic salmon and brown trout (*Salmo trutta*) was 19 400 kg during 1974–83. Brown trout constituted about 600 kg/yr. Mean weight of individual salmon was 8.1 kg and of trout was 0.6 kg (Heggberget et al. 1984). Spawning areas of salmon and trout are well distributed over the region of the river supporting salmon (Heggberget et al. 1984). Only sport fishing is allowed in this river, and no stocking of salmon fry or smolts has been reported in the Alta during the last 20 yr. A number of other fish species exist in the Alta but together constitute only about 2% of the total catch by electrofishing (Heggberget et al. 1984).

For purposes of analysis, the river was divided into three sections (Fig. 1), each of about 10–15 km. Section 1 lies nearest the river mouth and has relatively calm water. Section 2, the middle region of the river supporting salmon, consists of evenly dispersed pools and rapids. Sections 2 and 3 are separated by a small waterfall which adult salmon apparently negotiate without difficulty. The river in section 3 is similar to that in section 2.

Materials and Methods

Between 1981 and 1983, 3627 presmolt salmon were collected by electrofishing from the same localities (Fig. 1) in the three different sections, three times a year (July, August, and September). Scale analysis was used in determining the age of each fish, and the average length was calculated for each age group.

Scales from adult salmon were obtained from sport fishermen in the Alta. For each fish, the site, date of catch, sex, and size (length/weight) were recorded. Minor differences in sex distribution between the different parts of the river were noted.

Via scale analysis, one can analyse growth patterns (smolt age and smolt length) of adult salmon that were caught in different sections of the river. Young salmon that have grown quickly will have a lower smolt age than fish that have grown

TABLE 1. Mean length ($\bar{x}$, mm), standard deviation (SD), and number of fish within each age group (N) from three sections (S1, S2, S3) of the River Alta, 1981, 1982, and 1983.

		0+		1(+)		2(+)	
Period	Section	$\bar{x}$±SD	N	$\bar{x}$±SD	N	$\bar{x}$±SD	N
22–28 July 1981	S1	30±2	37	57±6	84	75±8	15
	S2	30±1	88	52±5	49	76±6	13
	S3	35±3	2	70±5	82	95±8	48
15–18 Aug. 1981	S1	37±3	113	65±5	81	80±5	14
	S2	33±3	105	62±5	49	89±7	59
	S3	43±3	11	78±5	27	99±6	19
08–12 Sept. 1981	S1	41±3	76	71±6	30	95±10	12
	S2	35±3	88	63±6	17	92±10	39
	S3	46±4	26	77±5	66	105±12	31
20–23 July 1982	S1	29±2	34	53±6	82	78±8	11
	S2	29±1	110	50±7	51	79±7	23
	S3	30±1	21	58±6	44	89±6	45
18–20 Aug. 1982	S1	38±2	38	65±6	48	84±6	14
	S2	34±2	84	59±5	43	86±6	27
	S3	40±3	81	71±6	31	103±7	22
22–25 Sept. 1982	S1	43±4	35	64±7	7	78±4	4
	S2	35±3	41	62±4	14	88±8	19
	S3	46±4	16	74±7	10	120±11	17
18–21 July 1983	S1	33±2	28	57±5	94	81±7	50
	S2	31±1	87	54±4	51	79±6	32
	S3	32±2	45	66±5	49	93±6	39
15–18 Aug. 1983	S1	42±3	111	65±5	72	83±7	32
	S2	33±3	45	58±6	33	81±8	38
	S3	38±3	54	68±4	59	97±8	33
12–15 Sept. 1983	S1	47±3	68	65±6	45	88±8	15
	S2	36±4	67	58±5	36	84±8	39
	S3	41±4	57	71±6	65	102±13	30

slowly (Elson 1962). The transition from growth in freshwater to growth in salt water is easily seen through analysis of scales from individual fish. The average smolt age for fish from different sections in the Alta is therefore a good indicator of how quickly the fish have grown in freshwater. The adult salmon scale sample consisted of 59 fish in 1981, 200 fish in 1982, and 318 fish in 1983, or a total of 577 adult fish.

Possible differences in growth among the three groups of fish were tested by analysis of variance with the Scheffé a posteriori test to reveal the sources of variance in cases where significant ($P < 0.05$) differences were found (Scheffé 1959).

In July 1981 an additional sample of presmolt fish was collected for electrophoretic analysis from each of the three sampling areas. Fish lengths varied between 4 and 12 cm in these samples, indicating that mainly 1- and 2-yr-old parr were included. The fish were immediately frozen and stored at −25°C for 2 mo until the electrophoretic analysis was conducted. Tissue samples of eye, liver, and muscle were extracted and horizontal starch–gel electrophoresis was performed following the methods of Utter et al. (1974). Staining procedures, interpretation of electrophoretic banding patterns, and designation of loci have been described previously by Allendorf et al. (1977), Cross and Ward (1980), and Ståhl (1981).

The following 19 enzymes were examined (the corresponding 37 loci scored are given in parentheses): aspartate aminotransferase (*Aat-1,2,3*), alcohol dehydrogenase (*Adh*), α-glycerophosphate dehydrogenase (*Agp-1,2*), adenylate kinase (*Ak-3*), creatine phosphokinase (*Cpk-1,3*), diaphorase (*Dia*), glyceraldehyde-3-phosphate dehydrogenase (*Gapdh-2*), glutamate dehydrogenase (*Gdh*), β-glucoronidase (*Gus*), isocitrate dehydrogenase (*Idh-1,2,3*), lactate dehydrogenase (*Ldh-1,2,3,4,5*), malate dehydrogenase (*Mdh-1,2,3*), malic enzyme (*Me-1,2*,3), 6-phosphologluconate dehydrogenase (*6-Pgdh-1,2*), phosphoglucose isomerase (*Pgi-1,2*), phosphoglucomutase (*Pgm-1,2*), sorbitol dehydrogenase (*Sdh-1,2*), superoxide dismutase (*Sod*), and xanthine dehydrogenase (*Xdh*).

Results

Growth of Presmolts

It appears that young salmon in the upper part of the River Alta (section 3) have better overall growth than those inhabiting the river further down (Table 1). Differences among 0+ salmon were small, while differences between older age-classes from varying river stretches were greater. During most years and survey periods, young salmon from furthest downriver (section 1) grew faster than those from the middle (section 2). On the whole, 0+ fish from section 3 were 0.5–1 cm longer than those from sections 1 and 2, while the corresponding difference between older age-classes was 1–2 cm.

Growth of presmolts at section 3 were significantly better than at section 1 and 2 in seven of nine cases ($P < 0.05$). The two remaining cases revealed no significance of age group 0+

TABLE 2. Mean length ($\bar{x}$) of presmolt salmon from the three different sections (S1, S2, S3) of the River Alta. Differences analysed by analysis of variance and ranged by Scheffé procedure. Samples of analog sampling periods (July, August, September) of 1981–83 are pooled. *denotes pairs of groups significantly different at the 0.050 level.

		July			August			September		
Age	Section	$\bar{x}$ (*N*)	S1	S2	$\bar{x}$ (*N*)	S1	S2	$\bar{x}$ (*N*)	S1	S2
0+	S1	31 (99)		*	39 (262)		*	43 (179)		*
	S2	30 (285)			33 (234)			35 (196)		
	S3	32 (68)	*	*	39 (146)		*	43 (99)		*
1+	S1	56 (260)		*	65 (201)		*	67 (82)		*
	S2	52 (151)			60 (125)			60 (67)		
	S3	66 (175)	*	*	71 (117)	*	*	74 (141)	*	*
2+	S1	79 (76)			83 (60)			89 (31)		
	S2	78 (68)			85 (124)	*		88 (97)		
	S3	93 (132)	*	*	99 (74)	*	*	107 (78)	*	*

TABLE 3. Smolt age and distribution of smolt age (%) within the three sections studied. Smolt age based on analyses of scales from adult salmon; *N* = number of fish.

		Smolt age (yr)								
		3		4		5		6		Mean smolt
Year	Section	*N*	%	*N*	%	*N*	%	*N*	%	age
1981	S1	—	—	10	83.3	2	16.7	—	—	4.17
	S2	1	3.1	26	81.3	5	15.6	—	—	4.13
	S3	3	20.0	12	80.0	—	—	—	—	3.80
1982	S1	11	10.5	75	71.4	19	18.1	—	—	4.08
	S2	6	7.4	46	56.8	29	35.8	—	—	4.28
	S3	1	7.1	13	92.9	—	—	—	—	3.93
1983	S1	14	12.8	49	45.0	45	41.3	1	0.9	4.30
	S2	9	6.2	66	45.2	70	47.9	1	0.7	4.43
	S3	14	22.2	38	60.3	11	17.5	—	—	3.95

between section 3 and 1, while section 3 had better growth than section 2 in all cases examined (Table 2).

Concerning differences between growth levels of presmolt salmon in sections 1 and 2, age groups 0+ and 1+ in section 1 had significantly better growth in all six cases examined (Table 2), while 2+ fish in section 2 had significantly better growth than 2+ fish from section 1 in one of three cases examined (August). The material from July and September showed no significant length differences between 2+ salmon from section 1 and 2. A possible explanation of the differences between 2+ and the younger age groups in section 1 and 2 may be that the number of 2+ fish is too low (Tables 1 and 2). Considering all the age groups, fish from section 1 had significantly better growth ($P < 0.05$) than fish from section 2 in six of nine cases, while section 2 had better growth ($P < 0.05$) in one of nine cases examined.

In summary, presmolt salmon in the upper part of the Alta have better growth than fish from the two lower sections, while fish in the lowest section of the river have better growth than fish from the middle section.

Mean Smolt Age and Length based on Analysis of Adult Salmon Scales

In 1981, section 3 contained 20% 3-yr-old smolts, while there were very few 3-yr-old smolts in the two other sections that year (Table 3). In 1982, the quota of 3-yr-old smolts was approximately evenly divided among the three sections, while in 1983 the proportion of 3-yr-old smolts was greater in section 3 (22%) than in the other two sections (6–13%).

There were no 5-yr-old smolts in section 3 in 1981 or 1982, while a quota of 18% was found in 1983. In the other two sections, the 5-yr-old smolt quota varied between 15 and 50%, with the highest being in 1983.

Smolt age was lowest for all three years in section 3 (3.80–3.95 yr). Smolt age was highest in section 1 in 1981 (4.17 yr) and in section 2 in 1982 and 1983 (4.28 and 4.43 yr, respectively). Analysis of variance of the pooled data from the three sampling years yielded significant smolt age differences among the sections ($P < 0.001$). A posteriori tests (Scheffé procedure, $P < 0.05$) revealed that mean smolt age in section 3 (3.92 yr) was significantly smaller than in sections 1 (4.19 yr) and 2 (4.35 yr), while smolt age in section 1 was significantly smaller than in section 2.

The regular geographic variation in smolt age shows that salmon in the Alta have varying growth rates in the different parts of the river. These variations in growth are principally similar to those exhibited by presmolts (Tables 1 and 2).

Salmon enter the Alta and start their upstream migration mainly in June and July. To determine whether fish heading for the upper part of the river were caught during migration in the

TABLE 4. Mean lengths of smolts ($\bar{x}$, mm) of different age categories within the three sections studied. Mean lengths based on back-calculations of scale samples of adult salmon. SD = standard deviation; N = number of observations.

		Smolt age (yr)								
		3			4			5		
Year	Section	$\bar{x}$	SD	N	$\bar{x}$	SD	N	$\bar{x}$	SD	N
	S1	—	—	—	135	15	10	165	13	2
1981	S2	130	—	1	139	15	26	152	14	5
	S3	139	12	3	151	19	12	—	—	—
	S1	109	15	11	131	14	75	145	19	19
1982	S2	109	13	6	133	18	46	148	20	29
	S3	141	—	1	152	15	13	—	—	—
	S1	111	14	13	132	18	46	142	17	44
1983	S2	118	22	9	126	14	62	143	18	63
	S3	128	19	10	151	24	32	159	19	5

TABLE 5. Mean lengths ($\bar{x}$, mm) of smolts based on analyses of scale samples of adult salmon caught at three different sections (S1, S2, S3) of the River Alta, 1981–83. *denotes pairs of groups significantly different at the 0.05 probability level (analyses of variance, Scheffé procedure); N = number of observations.

	3-yr-old smolts				4-yr-old smolts				5-yr-old smolts			
	$\bar{x}$	N	S1	S2	$\bar{x}$	N	S1	S2	$\bar{x}$	N	S1	S2
S1	110	24			132	131			145	65		
S2	116	16			131	134			145	97		
S3	132	14	*	*	151	57	*	*	159	5		

lower part of the river, the material was divided in two groups for each year. The first group consisted of fish caught during the entire period (June 1 to August 31), while in the second group, fish caught in June were excluded. Only small differences were observed in smolt age between the two periods, indicating that few of the fish were caught during upstream migration.

Mean lengths of smolts of all age groups were high among salmon caught in section 3 in all years examined (Table 4). On average, smolts of similar age were 1–2 cm longer than those from sections 1 and 2.

Analysis of variance of the pooled data of the three sampling years yielded significant length differences between the sections with respect to 3-yr-old smolts ($P = 0.001$) and 4-yr-old smolts ($P < 0.001$) (Table 5). Due to the lower smolt age in section 3, the material consisted of only five 5-yr-old smolts from section 3. Analysis of variance revealed no significant differences in smolt lengths among the three sections ($P = 0.218$). However, a posteriori tests (Scheffé procedure, $P < 0.05$) revealed that mean smolt lengths of 3- and 4-yr-old smolts were significantly longer in section 3 than in sections 1 and 2.

Genetic Variation of Presmolts

Genetic variation was observed at the following three loci (with the alleles segregating given in parentheses): *Aat-3* (*100, 50;25*), *Me-2* (*100, 125*), *Sdh-1* (*100, −50*). Allele frequencies (Table 6) were calculated by "allele counting" at *Aat-3* and *Me-2* whereas the frequency of the *Sdh-1* (*100*) allele was estimated from the square root of the proportion of *Sdh-1* (*100/100*) homozygotes.

Contingency chi-square tests for homogeneity revealed statistically significant allele frequency differences among locations at *Aat-3* and *Me-2* (Table 6), and the sum of chi-squares from the three loci is also significant ($\chi^2 = 34.3$, df = 6, $P < 0.001$). At *Me-2* and *Sdh-1* the same alleles were found in all samples whereas the two variant alleles at *Aat-3* were observed in the sample from section 2 only (Table 6). In contrast with the highly significant allele frequency differences, the estimates of average heterozygosity (H) are very similar for the three locations (Table 6), with a mean of 0.024.

The genotypic distributions within samples show no statistically significant deviations from Hardy–Weinberg expectations (Levene 1949) or from gametic phase equilibrium (Hill 1974); this is consistent with a model assuming that the samples represent populations in genetic equilibrium. Pairwise genetic distances (D) were calculated according to Nei (1972). The D-values (based on 37 loci) observed are of the same magnitude as those that have been previously reported for Atlantic salmon samples from different locations within a river (Ståhl 1981; Ståhl et al. 1983). The sample from section 2 in the central part of the river appears to be the most divergent one (cf. Table 6), and the difference between this location and those from sections 1 and 3 is larger than between the latter sections ($D = 0.0004$ between sections 1 and 2, 0.0005 between sections 2 and 3, and 0.0001 between sections 1 and 3).

Discussion

Analysis of mean lengths of individual year-classes of pre-

TABLE 6. Allele frequencies at variable loci and average heterozygosity estimates (H) based on 37 loci. Variant alleles were pooled when testing for allele frequency homogeneity at *Aat-3*; df = degrees of freedom.

Locality	Number of fish	*Aat-3*			*Me-2*		*Sdh-1*		H (%)
		100	50	25	100	125	100	−50	
Section 1	121	1.0	0.0	0.0	0.318	0.682	0.328	0.672	2.4
Section 2	104	0.938	0.057	0.005	0.212	0.788	0.340	0.660	2.4
Section 3	80	1.0	0.0	0.0	0.319	0.681	0.387	0.613	2.5
χ^2 (df=2)		25.67 ($P < 0.001$)			7.73 ($P < 0.025$)		0.87 ($P > 0.50$)		

smolt Atlantic salmon caught in three different sections of the River Alta showed that the fish from the upper part of the river grew more quickly than fish from the two lower sections. Fish from the lowest part of the river grew better than fish from the middle section in most of the cases examined. For fish older than 1 yr, the growth in the upper part of the river was significantly better (analysis of variance, $P < 0.05$) in each of the three years examined. The 0+ fish from the three sections had smaller growth differences than older fish, indicating that the growth differences between presmolts cannot be explained by earlier hatching in the upper than in the lower part of the river.

The time at which salmon becomes smolts is primarily dependent on size and not age (Elson 1962). Young fish that grow quickly will become smolts at a lower age than young fish that grow more slowly. The significantly lower smolt age of adult fish caught in the upper section of the Alta demonstrates that these fish have grown more quickly as presmolts than young fish from other sections of the river. In studying migration patterns of groups of fish from different river sections, one may use growth variations as biological tags instead of more traditional mechanical tags. The advantages are obvious in that no handling of fish is necessary. It is well known that varying handling and tagging methods, especially of small fish, may lead to changes in behavior patterns and increase mortality (Tilzey 1977; Jensen and Berg 1977).

Young hatchery-reared Atlantic smolts tend to return as multi-sea-winter salmon (Gardner 1976; Hansen and Lea 1982). If large salmon have a tendency to migrate furthest up river, this may mean that the fast-growing smolts from all sections migrate as spawners to the upper part of the river. If this is the situation in the Alta, then the growth differences used as biological tags cannot be used. However, the size of salmon caught by sport fishing in the different sections of the Alta was not found to be significantly different (analysis of variance, $P < 0.05$).

Several existing studies of growth in presmolt Altantic salmon indicate that growth differences among fish from the same river are not uncommon, even among fish living in relatively small rivers (Lund and Heggberget 1985). Randall (1982) reported that highest growth rates among Atlantic salmon in a river were found in areas of higher water temperature and low population density. Egglishaw and Shackley (1977) found similar correlations between growth and environment in several rivers in Scotland. There is no similar correlation between growth and population density in the Alta. The upper stretch of the Alta has both higher population density and better growth of young fish than the middle and lower sections (Heggberget et al. 1984). Better growth among young salmon living in the upper section of the river is probably attributable to that section's connection with an inflowing lake, a factor leading to increased direct and indirect sources of nourishment and to delayed cooling of the water throughout the autumn. However, a genetic contribution to differences in growth cannot be ruled out.

Based on scale analysis, adult salmon caught in the upper section of the Alta had faster growth and lower smolt age than fish caught in the middle and lower sections. The differences of growth pattern between the upper and lower sections were similar for each of the three years studied. This indicates that the results are not incidental or caused by variations of physical factors from year to year. There are apparently no other studies on growth patterns of adult salmon caught in different parts of the same river.

The correlation between river growth demonstrated for presmolt salmon and recaptured adult salmon supports the hypothesis that young salmon that spend their first years in the upper part of the river return to that area as adults. It is not known whether salmon stay in this area until spawning. However, there are no reported observations of salmon migrating downstream before spawning. It is therefore highly probable that salmon stay in this area until spawning. This gives reason for suggesting that the migration system shown in the Alta may be responsible for maintaining local populations within the river.

The highly significant allele frequency differences observed among the river sections indicate the existence of local populations with restricted gene flow between them. Homing to a particular part of the river constitutes an apparent basis for the development and maintenance of such a system of geographically separated and genetically differentiated subpopulations within a river. It is obvious that the location that is most divergent morphologically (section 3) is not the one that is most divergent genetically (section 2). It must be stressed, however, that such a direct coupling between genetic and morphologic divergence is not to be expected from the present data. First, there is no information to what extent the growth differences reflect genetic differences at loci determining growth rate and age at smoltification. Second, there is no reason to assume that different evolutionary forces such as natural selection and genetic drift have identical effects on electrophoretic loci and those directly affecting morphological characters.

Many investigations (Foerster 1936; White 1936; Dahl 1939; Ball 1955; Stuart 1957; Leggett 1977; Quinn and Fresh 1984) have shown that salmon find their way back to the river of their origin with relatively high precision; however, there is little detailed documentation of homing within one river course, especially in Atlantic salmon. Recent information indicates that olfaction is largely responsible for homing among Atlantic salmon, and much evidence suggests that this ability is genetically linked.

There exist several reports describing migrating patterns

within river systems of Pacific salmon (*Oncorhynchus* spp.) (Foerster 1936; Donaldson and Allen 1957; Jensen and Duncan 1971; Quinn and Fresh 1984), steelhead trout (*Salmo gairdneri*) (Wagner 1969), and Arctic char (*Salvelinus alpinus*) (Black and Dempson 1986). None of the results from these studies are contradictory to the hypothesis of local homing of Atlantic salmon in the Alta. Donaldson and Allen (1957) analysed the migration pattern of tagged coho salmon (*O. kisutch*), released as fingerlings from different hatcheries in the drainage system of Lake Washington. They found that the salmon not only returned to their native stream, but almost exclusively to the sites of release (194 of 195 fish). Shapovalov and Taft (1954) reported lower homing rates (85 and 73%) in a study of wild coho salmon in two small California streams.

Quinn and Fresh (1984) studied homing and straying in chinook salmon (*O. tshawytscha*) within the Colombia River system. By releasing tagged fish from a hatchery in one of the tributaries to the Colombia River, they were able to calculate the degree of homing. A very high proportion of the recaptures (98.6%) was made in the tributary where the fish were released, and very close to their hatchery. Most of the other recaptures were made further upstream in the Columbia River, while only 1.7% of the strays were caught in freshwater outside the Columbia River system.

Wagner (1969) investigated the effect of stocking location of juvenile steelhead trout within a stream and demonstrated that homing imprint was influenced to some extent by stocking sites within a stream.

Black and Dempson (1986) tested pheromone attraction in anadromous Arctic char by transplanting presmolts and spawners from the main river to a tributary where they were kept in cages. Most of the few adult char released in the tributary migrated back to the area of the main river where they were originally captured within a few days, in spite of possible pheromone attraction (Nordeng 1977) from the caged fish in the tributary. These results demonstrate the existence of within-river homing also in adult Arctic char.

The above-mentioned results from other anadromous species principally support the hypothesis presented for Atlantic salmon in the Alta. The results were based on fish that were artifically reared and released a short time before seaward migration. Patterns of homing and straying to hatcheries and natural spawning areas may differ (Quinn and Fresh 1984), but our River Alta study indicates a similar tendency of homing in wild Atlantic salmon as shown for hatchery-reared fish. The wild salmon in the Alta experienced the bouquet of organic odors (Hasler and Wisby 1951; Hasler et al. 1978) and/or the population-specific pheromones released by related fish (Nordeng 1971) for 3–5 yr before migrating to salt water. Hatchery-reared smolts experienced the water odors at the release point for a shorter time, depending on the distance between the hatchery and the release location and the age of the hatchery smolts. If the length of the imprinting period is of any significance for the local homing, a better homing in wild salmon than in hatchery-produced salmon would be expected.

White (1936) investigated migration patterns of tagged Atlantic salmon in the Apple River, Nova Scotia, and observed that female adults stayed in the same pool throughout the summer; some of the tagged females returned to exactly the same pool as second-time spawners the year after. Male salmon did not show similar tendencies of staying in the same pool. White's (1936) conclusion that fish that first stopped in a pool remained there until spawning substantiates our idea that salmon caught in the Alta during the fishing season would have stayed in that same part of the river until spawning had they not been caught.

The most comprehensive study of Atlantic salmon movements within a river was by Power and McCleave (1980) who used radio transmitters to track several adult salmon in the Penobscot River, Maine. One group of fish was imprinted as smolts on the upper part of the river, while a second group was imprinted on the river's head of tide. No consistent movement pattern was uncovered. Movement was erratic, with wandering both upstream and downstream, interspersed with position holding. Some behavioral differences were found between salmon imprinted as smolts to headwaters and those released in the tidal heads. There was a pronounced tendency for fish with a definite home area to return to that area as adults. Those fish that did show homing tendencies travelled a great deal both up and down the river (often several kilometres) before establishing themselves in the vicinity of their original home place. Power and McCleave (1980) concluded that it is impossible to establish this as a general behavior pattern for Atlantic salmon, given the lack of adequate studies for comparison. Reasons for the relatively slight homing behavior in the Penobscot River salmon may be that the fish were hatchery reared and had only short contact with river water before they migrated seaward (Power and McCleave 1980).

If local homing in river salmon is genetically linked (Behnke 1972; Allendorf et al. 1976; Ryman et al. 1979), it should be possible to disturb that system by introducing fish with unknown ancestry. Both the Apple (White 1936) and the Alta seem to be genetically undisturbed, as there are no reports of fish introductions. It is highly probable that distinct up-river populations that have strong homing tendencies exist in these two rivers. In the Penobscot investigations, hatchery-reared fish with unknown ancestry were planted in the river, and a system of population partitions has not had time to establish itself (Power and McCleave 1980); here, a reduced homing tendency was recorded.

When Pacific salmon from different river systems are crossbred, straying apparently increases (Bams 1976). One does not know the effects of eroding a local homing system within a river. If a local population exists within a river, it is reasonable to assume that natives are advantageously adapted to specific local environments. Until the consequences of possible crossbreeding are fully understood, one should endeavor to stock fish in the area of the river when their parents were originally captured.

Acknowledgements

We are deeply indepted to the director of the Alta Laksefiskeri Interessentskap, Mr. Osvald Möllenes, for his untiring assistance in organizing the collection of scale samples from adult salmon in the Alta River. This study has been financially supported by the Norwegian Hydroelectrical Board, The Directorate for Nature Management (Norway), and the Swedish Natural Science Research Council. Mrs. Amy Lightfoot is thanked for linguistic assistance.

References

ALLENDORF, F. W., N. MITCHELL, N. RYMAN, AND G. STÅHL. 1977. Isozyme loci in brown trout (*Salmo trutta* L.): detection and interpretation from population data. Hereditas 86: 179–190.

ALLENDORF, F. W., N. RYMAN, A. STENNEK, AND G. STÅHL. 1976. Genetic variation in Scandinavian brown trout (*Salmo trutta* L.): evidence of distinct sympatric populations. Hereditas 83: 73–82.

BALL, O. P. 1955. Some aspects of homing in cutthroat trout. Proc. Utah Acad. Sci. Arts Lett. 32: 75–80.

BAMS, R. A. 1976. Survival and propensity for homing as affected by presence or absence of locally adapted paternal genes in two transplanted populations of pink salmon (*Oncorhynchus gorbuscha*). J. Fish. Res. Board Can. 33: 639–671.

BEHNKE, R. J. 1972. The systematics of salmonid fishes of recently glaciated lakes. J. Fish. Res. Board Can. 29: 639–671.

BERG, M. 1964. Nord-Norske lakseelver. Johan Grundt Tanum Forlag, Oslo 300 s. (In Norwegian with English summary)

BLACK, G. A., AND J. B. DEMPSON. 1986. A test of the hypothesis of pheromone attraction in salmonid migration. Environ. Biol. Fishes 15 (3): 229–235.

CARLIN, B. 1968. Migration of salmon. Alt. Salmon Assoc. Spec. Publ. (Montreal, Que.) 14–22.

CLUTTER, R. I., AND L. E. WHITESEL 1956. Collection and interpretation of sockeye salmon scales. Bull. Int. Pac. Salmon Fish. Comm. 9: 159 p.

CROSS, T. F., AND R. D. WARD. 1980. Protein variation and duplicate loci in the Atlantic salmon, *Salmo salar* L. Genet. Res. Cambridge 36: 147–165.

DAHL, K. 1939. Homing instinct in salmon. Salmon Trout Mag. 94: 19–26.

DONALDSON, L. R., AND G. H. ALLEN. 1957. Return of silver salmon *Oncorhynchus kisutch* (Walbaum) to point of release. Trans. Am. Fish. Soc. 87: 13–22.

EGGLISHAW, H. J., AND P. E. SHACKLEY. 1977. Growth, survival and production of juvenile salmon and trout in a Scottish stream, 1966–75. J. Fish Biol. 11: 647–672.

ELSON, P. F. 1962. Predator–prey relationship between fish-eating birds and Atlantic salmon. Bull. Fish. Res. Board Can. 133: 87 p.

FOERSTER, R. E. 1936. The return from the sea of sockeye salmon (*Oncorhynchus nerka*) with special reference to percentage survival, sex proportions and progress of migration. J. Biol. Board Can. 3: 26–42.

GARDNER, M. L. G. 1976. A review of factors which may influence the sea-age and maturation of Atlantic salmon, *Salmo salar* L. J. Fish Biol. 9: 289–327.

HANSEN, L. P., AND T. B. LEA. 1982. Tagging and release of Atlantic salmon smolts (*Salmo salar* L.) in the River Rana, Northern Norway. Inst. Freshwater Res. Drottningholm 60: 31–38.

HARDEN JONES, F. R. 1968. Fish migration. Arnold, London. 325 p.

HASLER, A. D., A. T. SCHOLZ, AND R. M. HORRALL. 1978. Olfactory imprinting and homing in salmon. Am. Sci. 66: 347–355.

HASLER, A. D., AND W. J. WISBY. 1951. Discrimination of stream odors by fishes and relation to parent stream behaviour. Am. Nat. 85: 223–238.

HEGGBERGET, T. G., R. A. LUND, AND B. V. ROSVOLL. 1984. Konsesjonsundersøkelser i Alta-Kautokeinovassdraget 1981–1983. Fisk. Rapp. nr. 5-1984. DVF-Reguleringsundersøkelsene. (In Norwegian)

HILL, W. G. 1974. Estimation of linkage disequilibrium in randomly mating populations. Heredity 33: 229–239.

JENSEN, A. L., AND R. N. DUNCAN. 1971. Homing of transplanted coho salmon. Prog. Fish-Cult. 33: 216–218.

JENSEN, K. W., AND M. BERG. 1977. Growth, mortality and migrations of the anadromus char, *Salvelinus alpinus* L., in the Vardnes river, Troms, Northern Norway. Inst. Freshwater Res. Drottningholm 56: 70–80.

LEGGETT, W. C. 1977. The ecology of fish migrations. Annu. Rev. Ecol. Syst. 8: 285–308.

LEVENE, H. 1949. On a matching problem arising in genetics. Ann. Math. Stat. 20: 91–94.

LUND, R. A., AND T. G. HEGGBERGET. 1985. Growth analysis of presmolt Atlantic salmon, *Salmo salar* L., at three sections of a small Norwegian stream. Holarct. Ecol. 8: 299–305.

MØLLER, D. 1970. Genetic diversity in Atlantic salmon and salmon management in relation to genetic factors. Int. Atl. Salmon Found. Spec. Publ. 1: 5–29.

NEI, M. 1972. Genetic distance between populations. Am. Nat. 106: 283–292.

NORDENG, H. 1971. Is the local orientation of anadromous fishes determined by pheromones? Nature (Lond.) 233: 411–413.

1977. A pheromone hypothesis for homeward in anadromous salmonids. Oikos 28: 155–159.

POWER, J. H., AND J. D. MCCLEAVE. 1980. Riverine movements of hatchery reared Atlantic salmon (*Salmo salar* L.) upon returns as adults. Environ. Biol. Fishes 5 (1): 3–13.

QUINN, T. P., AND K. FRESH. 1984. Homing and straying in chinook salmon (*Oncorhynchus tshawytscha*) from Cowlitz River Hatchery, Washington. Can. J. Fish. Aquat. Sci. 41: 1078–1082.

RANDALL, R. G. 1982. Emergence, population densities and growth of salmon and trout fry in two New Brunswick streams. Can. J. Zool. 60: 2239–2244.

ROSSELAND, L. 1971. Årsberetning for 1970. Landbruksdept., Oslo. (In Norwegian)

RYMAN, N. 1983. Patterns of distribution of biochemical genetic variation in salmonids: differences between species. Aquaculture 33: 1–21.

RYMAN, N., F. W. ALLENDORF, AND G. STÅHL. 1979. Reproductive isolation with little genetic divergence in sympatric populations of brown trout (*Salmo trutta*). Genetics 92: 247–262.

RYMAN, N., AND G. STÅHL. 1981. Genetic perspectives of the identification and conservation of Scandinavian stocks of fish. Can. J. Fish. Aquat. Sci. 38: 1562–1575.

SCHEFFÉ, H. A. 1959. The analysis of variance. Wiley, New York, NY.

SHAPOVALOV, L., AND A. C. TAFT. 1954. The life histories of the steelhead rainbow trout (*Salmo gairdneri gairdneri*) and silver salmon (*Oncorhynchus kisutch*). Calif. Fish Game Fish Bull. 98: 375 p.

STÅHL, G. 1981. Genetic differentiation among natural populations of Atlantic salmon (*Salmo salar*) in northern Sweden. *In* N. Ryman [ed.] Fish gene pools. Ecol. Bull. (Stockholm) 34: 95–105.

1983. Differences in the amount and distribution of genetic variation between natural populations and hatchery stocks of Atlantic salmon. Aquaculture 33: 23–32.

STÅHL, G., E. J. LOUDENSLAGER, R. L. SAUNDERS, AND E. J. SCHOFIELD. 1983. Electrophoretic study on Atlantic salmon populations from the Miramichi River (New Brunswick) system, Canada. ICES C.M. 1983 M:20.

STUART, T. A. 1957. The migrations and homing behaviour of brown trout (*Salmo trutta* L.). Freshwater Salmon Fish. Res. 18: 27 s.

THORPE, J. E., AND K. A. MITCHELL. 1981. Stocks of Atlantic salmon (*Salmo salar*) in Britain and Ireland: discreteness and current management. Can. J. Fish. Aquat. Sci. 38: 1576–1590.

TILZEY, R. D. J. 1977. Repeat homing of brown trout (*Salmo trutta* L.) in Lake Eucombene, New South Wales, Australia. J. Fish. Res. Board Can. 34: 1085–1094.

UTTER, F. M., H. O. HODGINS, AND F. W. ALLENDORF. 1974. Biochemical genetic studies of fishes: potentialities and limitations, p. 213–238. *In* D. C. Malins and J. R. Sargent [ed.] Biochemical and biophysical perspectives in marine biology. Academic Press, New York, NY.

WAGNER, H. H. 1969. Effect of stocking location of juvenile steelhead trout, *Salmo gairdnerii*, on adult catch. Trans. Am. Fish. Soc. 98 (1): 27–34.

WHITE, H. C. 1936. The homing of salmon in Apple River. J. Biol. Board Can. 2: 391–400.

Spatial Variability in Sedimentary Algal Microfossils and Its Bearing on Diatom-Inferred pH Reconstructions

Sushil S. Dixit and R. Douglas Evans

Trent Aquatic Research Centre, Trent University, Peterborough, Ont. K9J 7B8

Dixit, S. S., and R. D. Evans. 1986. Spatial variability in algal microfossils and its bearing on diatom-inferred pH reconstructions. Can. J. Fish. Aquat. Sci. 43: 1836–1845.

Spatial variability of algal microfossils was investigated in 20–21 surface (0–0.25 cm) sediment samples from Hannah and Clearwater lakes, Ontario, together with the variability in 20 downcore diatom-inferred pH profiles from Hannah Lake. Strong habitat-dependent spatial variability in the distribution of microfossils was observed in lakes of simple morphology. The abundance of planktonic taxa was high in deepwater sediments in both the lakes, while benthic taxa were common only in the sediments of shallow waters of Clearwater Lake. It is hypothesized that greater circulation and presence of sufficient light at all depths allowed benthic diatoms to flourish even in samples from the deep areas of Hannah Lake. The use of pH indicator diatom assemblages rather than individual taxa greatly reduced the variability in computing diatom-inferred pH. The variations were negligible within the samples collected from greater water depths. Results suggest that a single paleo-pH profile will provide an indication of the direction in change in pH, but that absolute pH results can be obtained only by utilizing several cores from many depth strata. The ability of index α and index B to accurately predict recent pH shifts of Hannah Lake was impaired because of its very acidic nature (pH < 4.5) in the past.

Les auteurs ont étudié la variabilité spatiale d'algues microfossiles de 20 et 21 échantillons de sédiments de surface (0–0,25 cm) provenant des lacs Hannah et Clearwater (Ontario) ainsi que la variabilité des profils de pH déduits à partir des diatomées de 20 carottes prélevées dans le lac Hannah. Les lacs de morphologie simple présentaient une variabilité spatiale de la distribution des microfossiles qui était fortement dépendante de l'habitat. L'abondance des taxons planctoniques était élevée dans les sédiments d'eaux profondes des deux lacs tandis que les taxons benthiques n'étaient courants que dans les sédiments des eaux peu profondes du lac Clearwater. Les auteurs formulent l'hypothèse que la circulation plus importante et la présence de suffisamment de lumière à toutes les profondeurs ont permis aux diatomées benthiques de croître, même dans les zones profondes du lac Hannah. L'utilisation de groupes de diatomées indicateurs du pH, plutôt que de taxons individuels, a permis de fortement réduire la variabilité lors du calcul du pH à partir des diatomées. La variabilité des échantillons prélevés aux profondeurs plus importantes était négligeable. Les résultats obtenus portent à croire qu'un seul profil du paléo-pH permet d'obtenir un indice de la direction de la variation du pH, mais que des valeurs absolues ne peuvent être obtenues qu'en utilisant plusieurs carottes provenant de plusieurs strates profondes. La forte acidité antérieure du lac Hannah (pH < 4,5) a empêché de déterminer avec précision les écarts récents du pH à l'aide des indices α et B.

Received October 10, 1985
Accepted May 30, 1986
(J8486)

Reçu le 10 octobre 1985
Accepté le 30 mai 1986

In the absence of past water chemistry data, there has been great interest among paleolimnologists in using algal microfossils to predict historical changes in lake pH (Davis and Berge 1980; Del Prete and Schofield 1981; Dixit 1983; Flower and Battarbee 1983; Smol et al. 1984a; Charles 1985). Diatoms have been the most comprehensively studied group for this purpose primarily because of their ecological diversity, good preservation, and abundance in the sediments, together with recent advances in diatom taxonomy (e.g. Flower and Battarbee 1985; Charles 1986; Camburn and Kingston 1986). Diatoms occupy a variety of habitats in lakes, with many species being either benthic or euplanktonic/tychoplanktonic. Attention has also been focused recently on scaled chrysophytes, particularly members of the genera *Mallomonas* and *Synura* which are primarily euplanktonic (Smol et al. 1984a).

Meriläinen (1971) stated that the success of paleoecological interpretations depend on three essential conditions: (1) the microfossils accumulated in sediments should come from the lake ecosystems, (2) the preservation of the remains should be reasonably good, and (3) the remains of the different ecological communities should be completely mixed before final deposition. With respect to the third condition, in general it has been assumed that in a moderate-sized lake, the resuspended sediments from the shallow areas are directed towards the deepest part of the lake where they mix well with the pelagic sediments. Thus, a core taken from this area should provide an integrated sample representing both the littoral and pelagic regions (Brugam 1978; Fritz and Carlson 1982). Consequently, in virtually all pH reconstruction studies the interpretation of a lake's pH history has been based on a single sediment core taken from or near the deepest point of the lake.

The representative nature of a single diatom sample in meromictic lakes has been questioned by Meriläinen (1969, 1971). However, because of differences in circulation patterns and differential dissolution of siliceous frustules in monimolimnia of Meriläinen's lakes do not necessarily explain the distribution

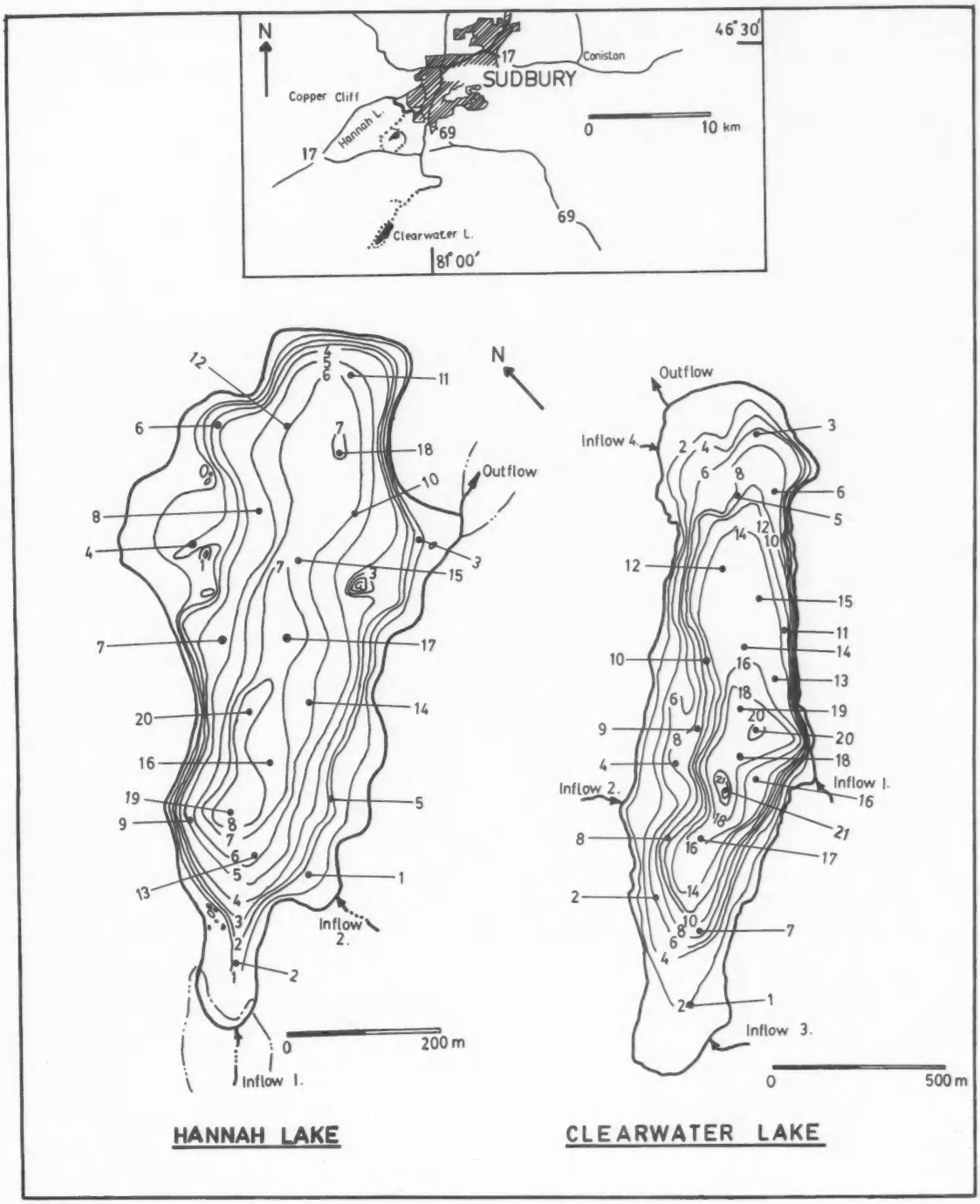


FIG. 1. Location and morphometry of Hannah and Clearwater lakes with the position of sampling sites.

TABLE 1. Selected physical and chemical characteristics of Clearwater and Hannah lakes. The pH values used are the mean of 12 measurements made by the Ontario Ministry of the Environment for the period August 1983 to July 1984.

Characteristics	Clearwater Lake	Hannah Lake
Surface area (ha)	76.5	23.7
Maximum depth (m)	21	8
Elevation (m)	302	306
Watershed area (ha)	340	76
pH	4.56	6.96
Alkalinity (μeq./L)	−41	310
Conductivity (μS/cm at 25°C)	76.4	308.4
Secchi depth (m)	10	4.5
Total P (μg/L)	5	7.9
Total N (μg/L)	35.5	84

of diatoms and chrysophytes in polymictic or dimictic lakes. In large complex basins, it has been found repeatedly that littoral flora are underrepresented in cores from the centre and overrepresented in cores from near the shore (Round 1964; Duthie and Sreenivasa 1972; Bradbury and Winter 1976).

The purpose of this study was to evaluate the spatial variability across a lake bottom in algal microfossils, more specifically in subfossil diatoms of lakes with simple morphometry. The role of such variability in interpreting reconstructions of lake acidification is examined in detail.

Study Lakes

Hannah and Clearwater lakes, situated about 5 and 13 km southwest of Sudbury, Ontario, were selected for this study (Fig. 1). The lakes are located on the granitic and siliceous rocks of the Canadian Shield. Measurements for August 1983 to July 1984 (n = 12) of Hannah and Clearwater lakes indicate a mean pH of 6.96 and 4.56, respectively (Ontario Ministry of the Environment (MOE), unpubl. data). Hannah Lake is holomictic, staying thermally homogenous at all times of year (MOE 1982), with a maximum depth of 8 m (Table 1). As a result of manipulations, this lake has undergone dramatic changes in lake water pH over the past two decades (MOE 1982). These changes have been well documented and provide background data to which the diatom-inferred pH history can be compared. Clearwater Lake is dimictic with a maximum depth of 21 m.

Materials and Methods

In July 1984, short sediment cores (approximately 20–25 cm) from different water depths (Fig. 1) of Hannah (n = 20) and Clearwater (n = 21) lakes were collected using a Kajak–Brinkhurst (K-B) gravity corer (Wilco Co. model 2402, Saginaw, MI). The corer was modified to hold Plexiglas coring tubes of 6.3 cm internal diameter, a size that has been shown to be reliable in obtaining undisturbed cores of recent sediments when compared with cores collected by divers (Evans and Lasenby 1984). On shore, cores were extruded and sectioned at intervals ranging from 0.25 to 0.50 cm for the top 3 cm of each core using Cross's (1977) technique. The surface sediment samples (0–0.25 cm) of both lakes and the remaining 2.75 cm of sediments of Hannah Lake were prepared for diatom and chrysophyte identification and counting following Dixit (1983). Quantitative subsamples of cleaned material were placed in evaporation trays (Battarbee 1973) and permanent mounts were prepared using Hyrax mounting medium. For each sample a minimum of 600 diatom valves and the number of chrysophyte scales in the same fields were identified and counted at 1000× magnification using a Carl Zeiss microscope equipped with phase contrast. Diatom identifications were based on Hustedt (1939), Huber-Pestalozzi (1942), Cleve-Euler (1951–55), Patrick and Reimer (1966, 1975), Foged (1981), and Germain (1981). Nicholls (1982), Wee (1982), and Smol et al. (1984b) were principle references for chrysophyte taxonomy.

Diatom taxa of Hannah and Clearwater lakes were assigned a pH indicator value based on published pH indicator information (Hustedt 1939; Nygaard 1956; Meriläinen 1967; Patrick and Reimer 1966, 1975; Foged 1981; van Dam et al. 1981; Charles 1985; Dixit and Dickman 1986; and others) and their pH-related distribution in 30 Sudbury lakes (Dixit et al., unpubl. data). Grouping was primarily in accordance with Hustedt's (1939) categories with minor modifications as discussed in Dixit (1986). The pH indicator groups were quantified to compute diatom-inferred pH utilizing multiple regression coefficients, index B (Renberg and Hellberg 1982), and index α (Nygaard 1956) derived from the study of surface sediment diatoms from 30 Sudbury lakes (Table 2). The counts of chrysophytes were considered as a group to study the spatial pattern of these planktonic organisms relative to diatoms. No attempts have been made to use them in predicted pH calculations because their pH preference has not been established for Sudbury lakes.

Results and Discussion

Hannah Lake

A total of 136 diatom taxa were identified; of those, only 6 were common (i.e. more than 5% of the total diatom counts) in Hannah Lake's surface samples (Fig. 2). *Achnanthes minutissima*, a benthic taxon (Stockner 1971; Stockner and Armstrong 1971) of circumneutral to alkaline pH lakes (Patrick and Reimer 1966; Foged 1981; Dixit and Dickman 1986), was the most abundant diatom in the majority of surface samples (Fig. 2). *Anomoeoneis vitrea*, with a preference towards alkaline waters, was the second most common benthic taxon (Hustedt 1939). Among the remaining four common diatoms, *Frustulia rhomboides*, *Navicula mediocris*, and *Eunotia* spp. were benthic taxa with preferences for acidic waters, while *Cyclotella stelligera* was the only common euplanktonic/tychoplanktonic taxon. Wide controversy exists concerning the true planktonic status of the latter taxon. Alhonen (1972) grouped it as both benthic and planktonic, Sreenivasa and Duthie (1973) found it among the plankton in the lower Great Lakes region, and Stoermer (1977) and Charles (1985) considered it as euplankton. Stockner (1971) suggested that *C. stelligera* may commence growth in the littoral regions in spring, forming chains that are dispersed by wave action or general turbulence to become part of the plankton.

Relative chrysophyte scale counts were significantly lower than diatoms (Fig. 2), and only five taxa were found in Hannah Lake samples. Of these, *Mallomonas crassisquama*, *M. pseudocoronata*, and *Synura petersenii* were common. *Mallomonas crassisquama* is not correlated with pH, whereas *M. pseudocoronata* and *S. petersenii* prefer alkaline and acidic conditions, respectively (Smol et al. 1984b).

TABLE 2. Inferred pH regression equations derived from surface sediment diatom assemblages of 30 Sudbury lakes (Dixit et al., unpubl. data). Standard errors are for regressions between measured lake water pH and pH predicted by diatoms.

Equation	r^2	Standard error	p
(1) Index α pH = 6.48−0.99 log index α	0.88	±0.42	<0.0001
(2) Index B pH = 6.75−1.18 log index B	0.88	±0.42	<0.0001
(3) Multiple reg. pH = 7.44−1.99 log ACB −0.019ACP −0.011CIR + 0.242 log ALP	0.93	±0.32	<0.0001

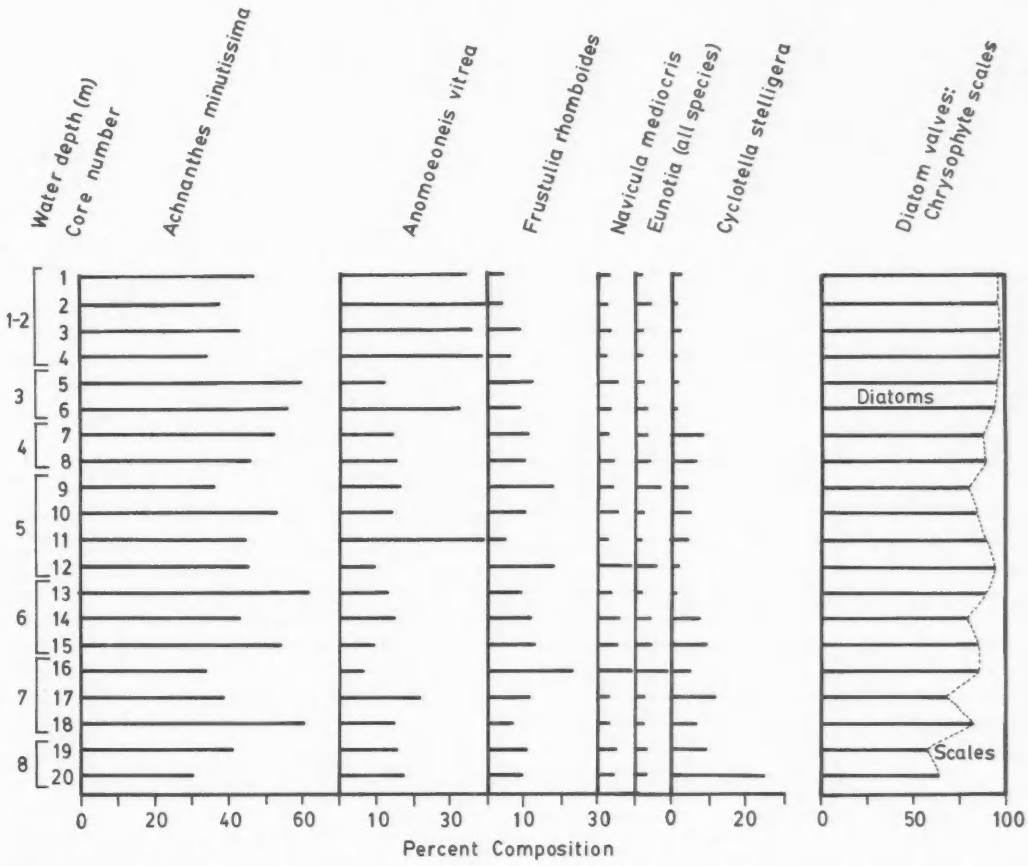


FIG. 2. Relative abundance of common diatom taxa and the ratio of diatom valves to chrysophyte scales of 20 surface sediment samples of Hannah Lake.

Depth-related gradients in the distribution of benthic taxa across the lake bottom were not clearly visible, but consistent with their planktonic nature, the percentages of *C. stelligera* and chryosophyte scales were higher in samples from greater water depths. Despite the lake's circumneutral pH (6.96), at which the planktonic diatom population should thrive (Almer et al. 1974; Charles 1985), benthic diatoms dominate, most probably because of its shallowness which has allowed benthic diatoms to grow at or near the area of maximum depth. The predominance of benthic forms in samples of <8 m water depth of Clearwater Lake (Fig. 3) also supports this hypothesis. The holomictic nature of Hannah Lake resulting in greater turbulance may have also played a significant role by allowing greater suspension, transportation, and redeposition of benthic diatoms from shallow to deeper parts of the basin than would occur in a dimictic lake where water circulation is less.

Clearwater Lake

In surface samples from Clearwater Lake a total of 104 diatom taxa were encountered. The common diatom taxa display strong spatial variability (Fig. 3). Benthic taxa (i.e. *F. rhomboides, Navicula bremensis, N. mediocris, Pinnularia*

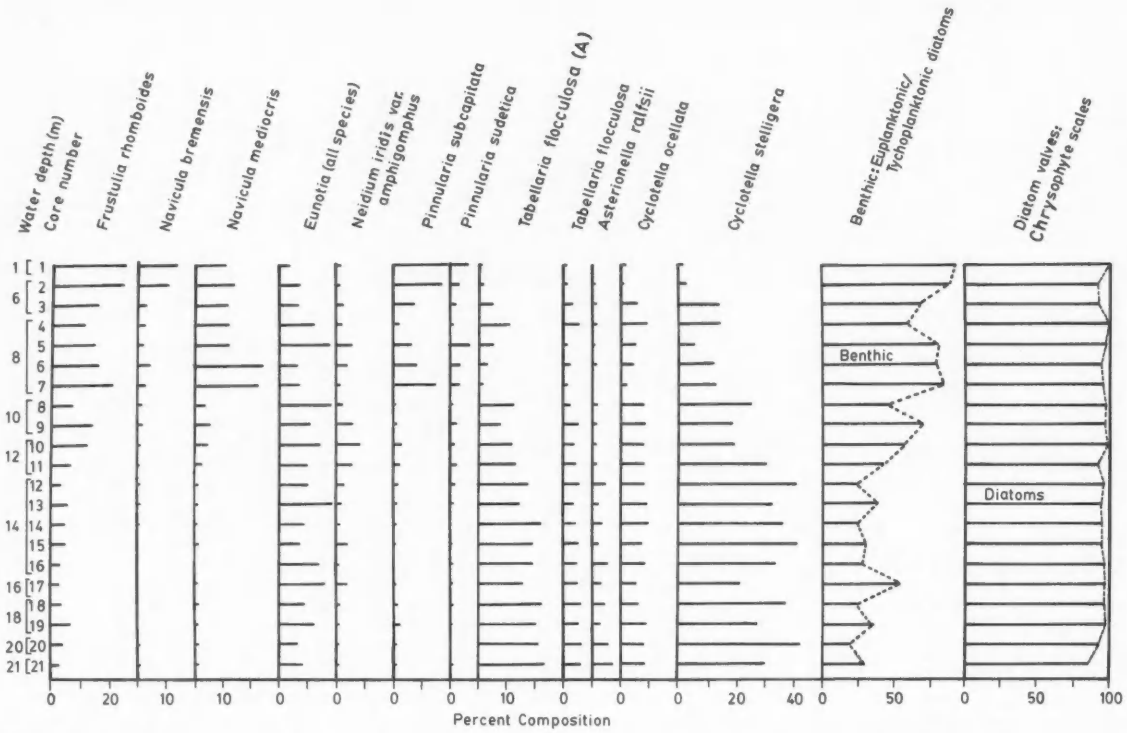


FIG. 3. Relative abundance of common diatom taxa and the ratio of diatom valves to chrysophyte scales of 21 surface sediment samples of Clearwater Lake.

subcapitata, and *P. sudetica*) were more common in surface sediment samples from ≤8 m water depths. However, at greater depths, lower numbers might be attributed to low light levels and correspondingly reduced growth. Planktonic taxa (i.e. *C. stelligera*, *Tabellaria flocculosa* A, *T. flocculosa*, and *Asterionella ralfsii* var. *americana*) increased in dominance with greater water depths (10–21 m). *Tabellaria flocculosa* designated as A (Fig. 3) is T. *flocculosa* Kütz. var. *flocculosa* strain IIIp (planktonic ecotype) sensu Koppen derived from perennial planktonic colonies (Koppen 1975). Similar to *C. stelligerra*, the nominate variety of *T. flocculosa* is tychoplanktonic (Meriläinen 1971; Stockner 1971). Although Charles (1985) has suggested that *A. ralfsii* var. *americana* is possibly euplanktonic, this has not yet been fully established. Because of the controversial status of certain taxa, in this study they are labelled either plankton or euplankton/tychoplankton. The presence of planktonic species in sediments of greater water depths of Clearwater Lake demonstrates that the commonly accepted hypothesis (Almer et al. 1974; Norton et al. 1981; Charles 1985) of planktonic diatoms disappearing when lake pH drops below 5.6 is not universally true.

The Clearwater Lake study strongly suggests that habitat-dependent preferential accumulation occurs for diatoms in different parts of the lake basin. The pattern closely follows the hypothesis proposed for complex lake systems (Meriläinen 1969; Bradbury and Winter 1976) that planktonic and tychoplanktonic (also termed metaphytonic by Round 1971) forms are overrepresented relative to benthic forms in sediment accumulated in greater water depths. This pattern may be influenced by the degree of steepeness of the lake bottom because frustules of planktonic diatoms, which are not attached to the bottom, will move easily downslope by turbulence to greater depths than frustules of benthic taxa, which are attached to the bottom. In addition, good growth of aquatic macrophytes in shallow areas also interferes with the movement of benthic forms (Meriläinen 1971).

Although Clearwater Lake has remained very acidic (pH < 4.6) over at least the past 15 yr, the relative abundance of individual acidobiontic taxa in surface samples never exceeded 4% of the total diatom counts. The acidobiontic taxa found were *Actinella punctata*, *Anomoeoneis serians*, *Eunotia exigua*, *Semiorbis hemicyclus*, and *Tabellaria quadriseptata*. Of Clearwater's 12 common diatom taxa, 10 are acidophilous (Fig. 3). Among the rest, *Cyclotella ocellata* is grouped as circumneutral and *C. stelligera* as pH indifferent. Along with its planktonic nature, widespread discrepancy also exists regarding the pH indicator status of *C. stelligera*. Cholnoky (1968) and Foged (1977) found this taxon alkaliphilous (pH optimum 7.5–8.0 and 7.6–8.9, respectively) whereas Meriläinen (1967), van Dam et al. (1981), and Charles (1985) found it common in circumneutral waters (they used the terms circumneutral and indifferent synonymously). Schofield and Galloway (1977) found it as common plankton (6–8% of the total diatom counts) in two acidic lakes (pH 4.7–5.3). Such diverse pH tolerances, as well as its presence in other Sudbury lakes of a wide pH range (unpublished data), strongly suggests to us that *C. stelligera* should be grouped with diatoms that have no specific pH preference.

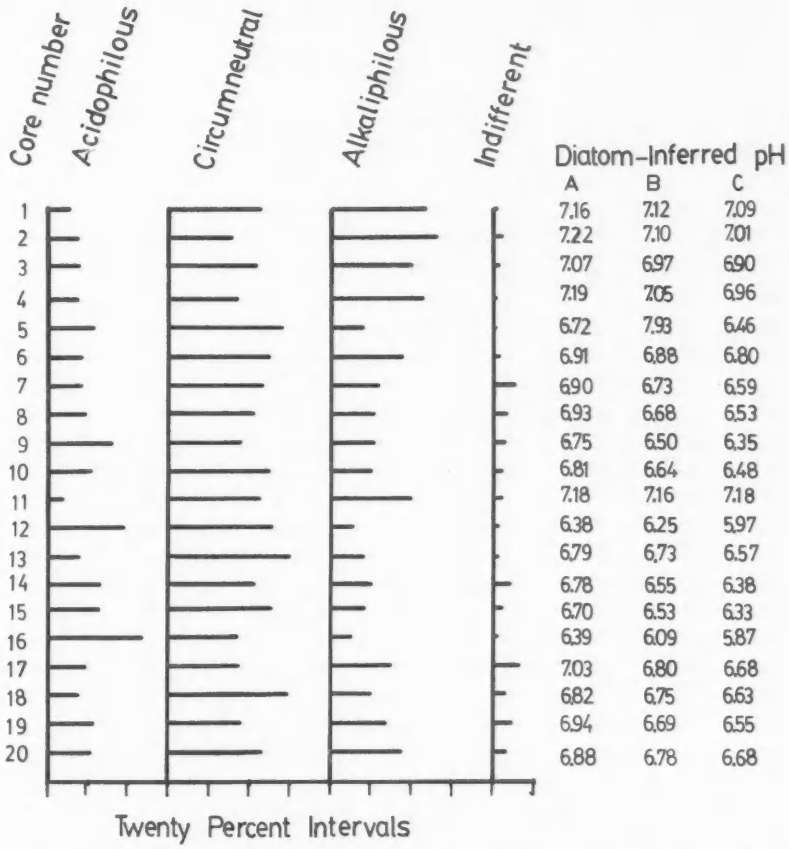


FIG. 4. Percent composition of pH indicator diatom groups and diatom-inferred pH of 20 surface samples of Hannah Lake. A, inferred pH using multiple regression method; B, inferred pH using index B; C, inferred pH using index α.

Relative to Hannah Lake, the ratios of chrysophyte scale counts to diatom frustules in Clearwater Lake were extremely low (Fig. 3). Nine chrysophyte taxa were identified; among them, *M. crassisquama, Synura echinulata, S. macracantha,* and *S. sphagnicola* were common. With the exception of generalist *M. crassisquama*, other common taxa are indicators of acidic waters (Smol et al. 1984b). Unlike Hannah Lake, there was no depth-related pattern in the distribution of these planktonic organisms. The relatively low scale counts (2–98 scales per sample) in Clearwater samples do not follow closely the study of Adirondack lakes (Smol et al. 1984a, 1984b) where chrysophyte scales were common in acidic lakes. The lake has been acidic (pH < 4.6) for at least 15 yr (MOE 1982), and this should be enough time to compensate for any lag period in a chrysophyte species shift, if this was needed. However, the high trace metal concentrations found in Clearwater Lake (MOE 1982) may be partially responsible for the poor chrysophyte population. Our ongoing study of chrysophytes in lakes of wide metal range may provide information on this little known subject.

Variability in Surface Sediment Inferred pH

The pH indicator diatoms of both lakes were grouped into various pH indicator categories and the diatom-inferred pH was computed for each surface sample (Fig. 4 and 5). In surface samples from Hannah Lake there was no depth-related pattern in the distribution of pH indicator diatom groups. As might be expected from a lake water pH of 6.96, the circumneutral diatom assemblage was most abundant followed by alkaliphilous and acidophilous assemblages (Fig. 4). Diatom-inferred pH variability results are discussed primarily with reference to the pH value obtained by the multiple regression of pH indicator diatom assemblages because of its overall superiority to the other two methods in the Sudbury (Table 2) and Algoma lake regions (Dixit 1986).

The inferred pH of Hannah Lake's samples ranged from 6.38 to 7.22, with 90% of inferences being within ±0.3 of the lake water pH. An area-weighted "whole-lake average" pH estimate was obtained by dividing the lake into depth strata and assuming that the average value of the samples for a given stratum typified the entire stratum. By summation, a whole-lake average was obtained. For Hannah Lake, the area-weighted inferred pH of 6.97 is almost identical to the mean measured pH of 6.96. The area-weighted pH was also better than the simple geometric mean of 20 inferred pH results (pH 6.88). Compared with multiple regression inferred pH, the variability was high in index α (5.87–7.18) and index B (6.09–7.12)

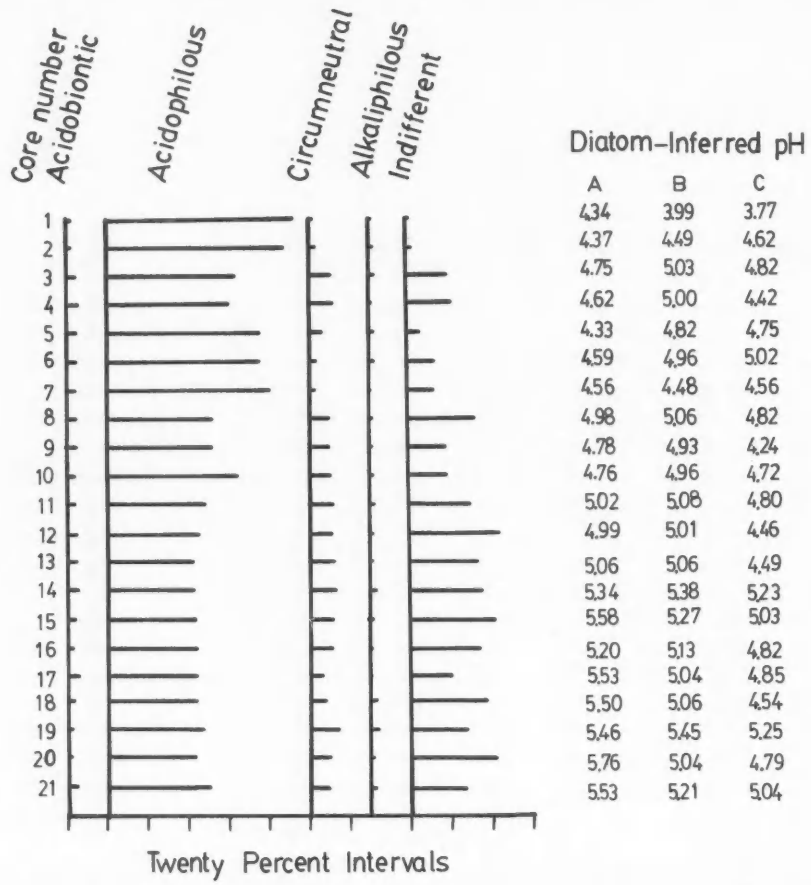


FIG. 5. Percent composition of pH indicator diatom groups and diatom-inferred pH of 21 surface samples of Clearwater Lake. A, inferred pH using multiple regression; B, inferred pH using index B; C, inferred pH using index α.

inferred pH results.

Similar to the common diatom taxa (Fig. 3), acidophilous and pH-indifferent diatom assemblages of Clearwater Lake also indicated a depth-related pattern (Fig. 5), but the gradient was less evident. This was because in the deeper water samples the benthic taxa were replaced by planktonic taxa of the same pH indicator value. The dominance of benthic acidophilous taxa in samples of ≤8 m water depths (cores 1–7) resulted in a higher percent composition of acidophilous diatom assemblages. However, the situation was different for the pH-indifferent assemblage because *C. stelligera* was the most dominant taxon in samples from >8 m (Fig. 3).

The range in inferred pH was 1.43, 1.46, and 1.48 pH units for multiple regression, index B, and index α, respectively. The slightly higher variability in index α and index B for both study lakes was due to oversensitivity of these two indices to acid or alkaline pH indicator assemblages (Charles 1985; Dixit 1986). The very high relative abundance of acidophilous diatoms (>95% of total diatom counts), resulting in an excessively low predicted pH for sample 1 (pH 3.99 and 3.77 by index B and index α, respectively), is a clear example of this. A definite pattern emerged of predicted pH from the multiple surface samples of Clearwater Lake. The predicted pH results of the samples from >8 m (4.76–5.76) were significantly higher than the samples from ≤8 m (4.33–4.75). The higher predicted pH of samples from the profundal zone is due to lower acidophilous diatom counts than in littoral samples. In deep water, the decline of the acidophilous assemblage was compensated by an increase in indifferent forms, more particularly by the planktonic *C. stelligera*. It is important to point out that the pH indicator value of planktonic taxa that occur in deepwater sediment samples will have a deciding effect on predicted pH results.

As in Clearwater Lake, the pH has also been overestimated in samples collected from deepwater locations of other acidic lakes (Fortescue et al. 1984). In the absence of historical lake pH data these results had been explained on the basis of a lag period which may be required by diatoms to accommodate any shift in lake water pH. The study of Clearwater Lake suggests an additional or alternative explanation because the lake has remained at its present pH level over the past 15 yr (MOE 1982). The difference may be due to the presence of certain planktonic diatoms that may be more sensitive to physical (i.e. water depth) or chemical characteristics other than pH.

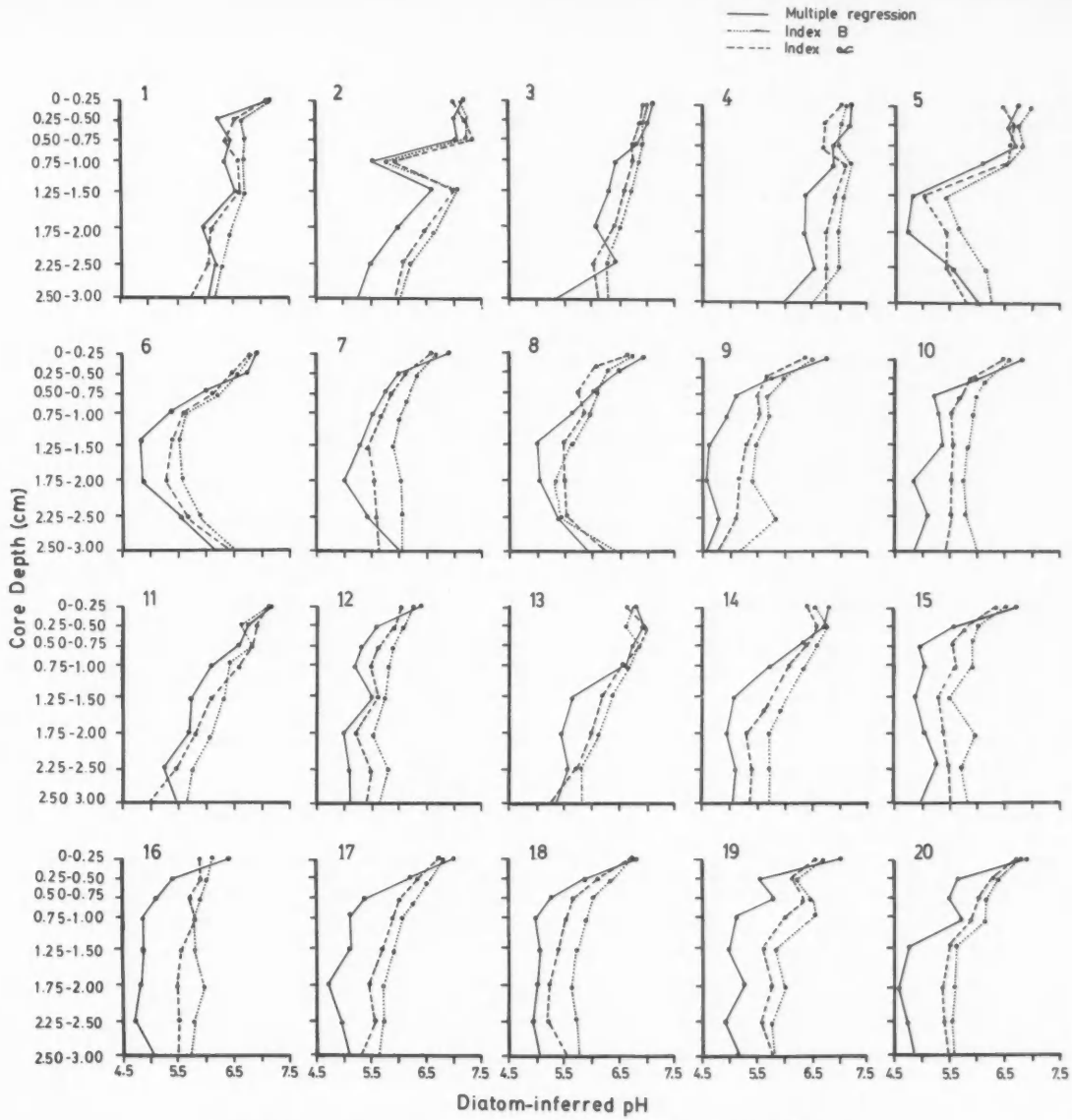


FIG. 6. Paleo-pH profiles of the top 3 cm sediments of 20 cores of Hannah Lake.

Cyclotella stelligera was a case in point, not only in Clearwater Lake but also in other lakes in the Sudbury region (Dixit et al., unpubl. data).

Although in Clearwater Lake the pH inferred for samples of >8 m depths was as much as 1.2 pH units greater than the observed lake water pH, as in Hannah Lake, the area-weighted inferred pH (4.63) was very close to the observed pH (4.56). The simple geometric mean of inferred pH (for Clearwater Lake 5.0) of multiple sediment samples would not have circumvented the spatial variability in surface samples as did the area-weighted technique.

Variability in Downcore Inferred pH

The representative nature of a single paleo-pH profile was tested by reconstructing the recent pH history of Hannah Lake in the top 3 cm of 20 sediment cores (Fig. 6). Measured pH of this lake in 1973–1974 averaged 4.31 (MOE 1982). In 1975 the lake was neutralized to pH 7; since then, pH has stabilized at about this level. In the centre of the lake, the sediment accumulation rate determined from ^{210}Pb dating is approximately 0.8 mm/yr (MOE 1982). The *Ambrosia* horizon from the same core suggested a comparable sedimentation rate (0.93 mm/yr). Thus, about 1 cm of sediment would have accumulated since 1975 and the neutralization effects should be mirrored by an increase in inferred pH in the cores.

Except for cores collected from 1–2 m water depths, all the cores showed a recent increase in lake water pH, supporting the pH indicator potential of sedimentary diatoms. However, there

were differences in the extent of these inferred pH changes both among the cores and also among the three techniques for inferring pH profiles for a single core. In interpreting downcore paleo-pH variability, only trends are considered because specific changes do not occur at the same depth in each core due to spatial variability in the sediment accumulation rate (Evans and Rigler 1983). Three different patterns of paleo-pH profiles are evident in Hannah Lake (Fig. 6). Cores (1–4) taken from shallow areas (1–2 m) failed to predict the lake's known pH record of the past 12 yr. This is most likely due to the greater rates of physical and biological perturbations which subsequently influence the chronological deposition of sediment in shallow areas of a lake basin. However, with the exception of core 11, there was an increase in the inferred pH in cores collected from water depths greater than 2 m. In the majority of cores this increase occurred between 0.75 and 1.5 cm sediment depth. Spatial variability in sediment accumulation can account for the differences in the depth at which the increase occurred. However, the 1 cm accumulation (based on MOE dates) falls within the range found here.

In addition to an increase in inferred pH towards the surface, cores 5–8 also indicate an increase in inferred pH in the past (i.e. at the 3 cm depth). These four cores suggest accelerated acidification of about 1 full pH unit over a period in which 1.25 cm of sediment accumulated (between 1.75 and 3.0 cm). This increase in pH at depth was not observed in the other cores (9–20). This is not likely a result of diatom variability, but rather higher rates of sediment accumulation in deep areas of the lake. It has been reported repeatedly that sediment accumulation rates are significantly higher in areas of greater water depths than in shallow areas (Evans and Rigler 1983; Davis et al. 1984), suggesting that a 3-cm section of cores from 3–4 m of water depth will represent a longer time scale than the cores from the deeper zones.

While the surface sediments accurately predicted an increasing pH (except for core 9), the inferred pH results of the lower portion did not correspond to the 1973–74 lake water pH of 4.3. In all cases the inferred pH was high, a situation closely resembling results of surficial sediment from deepwater stations in Clearwater Lake. In most cases the difference was even greater for the predictions of index B and index α (Fig. 6). This suggests that the predictive value of the diatom-inferred pH technique may become less at pH's below 5. Further research of other calibrated lakes would provide more conclusive information.

The comparison of index α and index B pH profiles with those based on multiple regression indicates that in the upper sediments of the cores (approximately 0–0.5 cm), when lake water pH was high their predictions were close to those of multiple regression (Fig. 6). Variability increased greatly with the sediment depth in the majority of cores, suggesting that when the lake pH is high, inferred pH derived from various indices may be in good agreement with present lake water pH, but when the lake pH is low, index α and index B may not accurately predict pH conditions. The multiple regression technique may suffer from some of the same problems, but appears to be superior in comparison with the other methods on the basis of its ability to predict Hannah Lake's known pH record.

Conclusions

This study indicates that specific habitat preference in algal microfossils and perhaps their postdepositional movement result in spatial variability even in small lakes. The extent of the spatial variability is different for holomictic (Hannah) and dimictic (Clearwater) lakes. Planktonic taxa occur more commonly in sediments obtained from deeper water depths whereas benthic taxa predominate in the sediments of shallow regions. The higher planktonic diatom counts and fewer chrysophyte scales in Clearwater Lake suggest that the commonly accepted hypotheses that planktonic diatoms are replaced in acidic lakes ($pH < 5.6$) by benthic forms and that chrysophyte scales occur in greater abundance in such lakes are not true for all cases. If this is due to regional differences, it will become evident from our ongoing detailed study of other Sudbury lakes.

In surface sediment samples the range in inferred pH was approximately 0.8 and 1.4 pH units for Hannah and Clearwater lakes, respectively, and the deepwater samples overestimated the observed pH in Clearwater Lake. The role of planktonic taxa in inferred pH calculations of such samples should be carefully evaluated. In both the lakes the similarity in area-weighted diatom-inferred and observed lake water pH strongly suggests that the use of samples from different parts of the lake when area weighted will provide the best estimate of diatom-inferred pH.

The comparison of downcore paleo-pH profiles with a lake's known pH history supports the pH indicator potential of sedimentary diatoms. However, there may be method-dependent problems when inferring pH of very acidic conditions. Hannah Lake's paleo-pH profiles are found to be replicable in areas where stable sediment accumulates (i.e. cores from >2 m water depth). When time and financial constraints are considered, a single paleo-pH profile produced from the deepest site of a lake will provide a reasonable trend of paleo-environmental perturbations. However, it must be emphasized that the results, while useful in explaining trends, will not yield absolute predictions of lake water pH.

Acknowledgements

This paper is a part of the Ph.D. thesis of S. Dixit to be submitted to Queen's University, Kingston. Financial support was made available in the form of a Queen's Graduate Fellowship to S.S.D. as well as a research contract from the Ontario Ministry of the Environment, and a research grant from the Natural Sciences and Engineering Research Council of Canada to R.D.E. Many thanks to Dr. A. S. Dixit for her help in the preparation of the manuscript and also to Mr. K. Walsh and Ms. M. Filo for their assistance in the field. Thanks are also due to the Ontario Ministry of the Environment for providing the lake pH data.

References

ALHONEN, P. 1972. The geological development and paleolimnology of a small polluted lake in S. Finland. Comment. Biol. 57: 1–34.

ALMER, B. W., W. DICKSON, C. EKSTRÖM, E. HANSTRÖM, AND V. MILLER. 1974. Effects of acidification on Swedish lakes. Ambio 3: 30–36.

BATTARBEE, R. W. 1973. A new method for the estimation of absolute microfossil numbers, with special reference to diatoms. Limnol. Oceanogr. 18: 647–653.

BRADBURY, J. B., AND T. S. WINTER. 1976. Areal distribution and stratigraphy of diatoms in the sediments of Lake Sallie, Minnesota. Ecology 57: 1005–1014.

BRUGAM, R. B. 1978. Human disturbance and the historical development of Linsely Pond. Ecology 59: 19–36.

CAMBURN, K. E., AND J. C. KINGSTON. 1986. The genus *Melosira* from soft-water lakes with special reference to northern Michigan, Wisconsin, and Minnesota, p. 17–34. *In* J. P. Smol, R. W. Batterbee, R. B. Davis, and J. Meriläinen [ed.] Diatoms and lake acidity. Junk, The Hague, The

Netherlands.

CHARLES, D. F. 1985. Relationships between surface sediment diatom assemblages and lakewater characteristics in Adirondack lakes. Ecology 66: 994–1011.

1986. A new diatom species, *Fragilaria acidobiontica*, from acidic lakes in northeastern North America. p. 35–44. *In* J. P. Smol, R. W. Batterbee, R. B. Davis, and J. Meriläinen [ed.] Diatoms and lake acidity. Junk, The Hague, The Netherlands.

CHOLNOKY, B. J. 1968. Die Ökologie der Diatomeen in Binnengewässer. J. Cramer, Lehre, Germany.

CLEVE-EULER, A. 1951–55. Die Diatomeen von Schweden und Finland. K. sv. Vet. Akad. Avh. Ser. IV; 961 p.

CROSS, P. M. 1977. An attempt to estimate phosphorus retention in Bob Lake from iron retention and iron:phosphorus ratio in the sediments. M.Sc. thesis, University of Toronto, Toronto, Ont. 1321 p.

DAVIS, M. B., R. E. MOELLER, AND J. FORD. 1984. Sediment focusing and pollen influx, p. 261–293. *In* E. Y. Haworth and J. W. G. Lund [ed.] Lake sediments and environmental history. University of Minnesota Press, Minneapolis, MN.

DAVIS, R. B., AND F. BERGE. 1980. Atmospheric deposition in Norway during the last 300 years as recorded in SNSF lake sediment, Part II. Diatom stratigraphy and inferred pH, p. 270–271. *In* D. Drabløs and A. Tollan [ed.] Ecological impact of acid preciptitation. Proc. Int. Conf. Ecol. Impact Acid Precip., SNSF Project, Oslo.

DEL PRETE, A., AND C. SCHOFIELD. 1981. The utility of diatom analysis of lake sediments for evaluating the acid precipitation effects on dilute lakes. Arch. Hydrobiol. 91: 333–340.

DIXIT, S. S. 1983. The utility of sedimentary diatoms as a measure of historical lake pH. M.Sc. thesis, Brock University, St. Catharines, Ont. 169 p.

1986. Diatom-inferred pH calibration of lakes near Wawa, Ontario. Can. J. Bot. 64. (In press)

DIXIT, S. S., AND M. D. DICKMAN. 1986. Correlation of surface sediment diatoms with the present lakewater pH in 28 Algoma lakes, Ontario, Canada. Hydrobiologia 131: 133–143.

DUTHIE, H. C., AND M. R. SCREENIVASA. 1972. The distribution of diatoms on the superficial sediments of Lake Ontario. Proc. 15th Conf. Great Lakes Res. 1772: 45–52.

EVANS, H. E., AND D. C. LASENBY. 1984. A comparison of lead and zinc sediment profiles from cores taken by diver and a gravity corer. Hydrobiologia 108: 165–169.

EVANS, R. D., AND F. H. RIGLER. 1983. A test of lead-210 dating for the measurement of whole-lake soft sediment accumulation. Can. J. Fish. Aquat. Sci. 40: 78–81.

FLOWER, R. J., AND R. W. BATTARBEE. 1983. Diatom evidence of recent acidification of two Scottish lakes. Nature (Lond.) 305: 130–133.

1985. The morphology and biostratigraphy of *Tabellaria quadriseptata* (Bacillariophyceae) in acid waters and lake sediments in Galloway, southwest Scotland. Br. Phycol. J. 20: 69–79.

FOGED, N. 1977. Freshwater diatoms in Ireland. Bibl. Phycol. 34: 221 p.

1981. Diatoms in New Zealand, the North Island. J. Cramer, Hirschberg, Germany. 225 p.

FORTESCUE, J., R. BARLOW, M. DICKMAN, J. TERASMAE, AND D. WADGE. 1984. Multidisciplinary followup of pH observations in lakes north and east of Lake Superior, District Algoma. Ont. Geol. Surv. Rep. 5342: 574 p.

FRITZ, S. C., AND R. E. CARLSON. 1982. Stratigraphic diatom and chemical evidence for acid strip-mine lake recovery. Water Air Soil Pollut. 17: 151–163.

GERMAIN, H. 1981. Flore des Diatomées. N. Boubée, Paris. 444 p.

HUBER-PESTALOZZI, G. 1942. Das Phytoplankton des süswassers. Die. Biennengevässer, Stuttgart. Band XVI: 549 p.

HUSTEDT, F. 1939. Systematsche und ökologische Untersuchungen uber die Diatomeen-Flora von Java, Bali und Sumatra. Archiv. Hydrobiol. Suppl. 16: 1–155, 274–394.

KOPPEN, J. D. 1975. A morphological and taxonomic consideration of *Tabellaria* (Bacillariophyceae) from the northcentral United States. J. Phycol. 11: 236–244.

MERILÄINEN, J. 1967. The diatom flora and hydrogen ion concentration of water. Ann. Bot. Fenn. 4: 51–58.

1969. Distribution od diatom frustules in recent sediments of meromictic lakes. Mitt. Int. Ver. Limnol. 17: 186–192.

1971. The recent sedimentation of diatom frustules in four meromictic lakes. Ann. Bot. Fenn. 8: 160–176.

MOE. 1982. Studies of lakes and watersheds near Sudbury Ontario: final limnological report. Ontario Ministry of the Environment, Toronto, Ont. SES 009/82.

NICHOLLS, K. H. 1982. *Mallomonas* species (chrysophyceae) from Ontario, Canada, including description of two new species. Nova Hedwigia 36: 89–124.

NORTON, S. A., R. B. DAVIS, AND D. F. BRAKKE. 1981. Responses of northern New England lakes to atmospheric inputs of acids and heavy metals. Land Water Resour. Cent. Proj. Rep. A-048-ME. University of Maine at Orono, Orono, ME. 90 p.

NYGAARD, G. 1956. Ancient and recent flora of diatoms and chrysophyceae in Lake Gribsø. Folia Limnol. Scand. 8: 32–99, 254–262.

PATRICK, R., AND C. W. REIMER. 1966. The Diatoms of the United States. Vol. 1. Monographs of the Academy of Natural Sciences No. 13, Philadelphia, PA. 688 p.

1975. The diatoms of the United States. Vol. 2, Part 1. Monographs of the Academy of Natural Sciences No. 13, Philadelphia, PA. 213 p.

RENBERG, I., AND T. HELLBERG. 1982. The pH history of lakes in southwestern Sweden, as calculated from the subfossil diatom flora of the sediments. Ambio 11: 30–33.

ROUND, E. E. 1964. The diatom sequences in lake deposits: some problems of interpretations. Verh. Int. Ver. Limnol. 15: 1012–1020.

1971. The growth and succession of algal populations in freshwaters. Mitt. Int. Ver. Limnol. 19: 70–99.

SCHOFIELD, C. L., AND J. GALLOWAY. 1977. The utility of paleolimnological analysis of lake sediments for evaluating acid precipitation effects on dilute lakes. Project Report A-071-N.Y. U.S. Department of Interior, Washington, DC. 23 p.

SMOL, J. P., D. F. CHARLES, AND D. R. WHITEHEAD. 1984a. Mallomonadacean microfossils provide evidence of recent lake acidification. Nature (Lond.) 307: 628–630.

1984b. Mallomonadacean (Chrysophyceae) assemblages and their relationships with limnological characteristics in 38 Adirondack (N.Y.) lakes. Can. J. Bot. 62: 611–630.

SREENIVASA, M. R., AND H. C. DUTHIE. 1973. The postglacial diatom history of Sunfish Lake, southwestern Ontario. Can. J. Bot. 51: 1599–1609.

STOCKNER, J. G. 1971. Preliminary characterization of lakes in the Experimental Lake Area, northwestern Ontario, using diatom occurrences in sediments. J. Fish. Res. Board Can. 28: 265–275.

STOCKNER, J. G., AND F. A. J. ARMSTRONG. 1971. Periphyton of the Experimental Lakes Area, southwestern Ontario. J. Fish. Res. Board Can. 28: 215–229.

STOERMER, E. F. 1977. Post-Pleistocene diatom succession in Douglas Lake, Michigan. J. Phycol. 13: 73–80.

VAN DAM, H., G. SUUROMOND, AND C. J. F. BRAAK. 1981. Impact of acidification on diatoms and chemistry of Dutch moorland pools. Hydrobiologia 83: 425–449.

WEE, J. L. 1982. Studies on the Synuraceae (Chrysophyceae) of Iowa. J. Cramer, Hirschberg, Germany, 183 p.

Effect of Stream Acidification on Periphyton Composition, Chlorophyll, and Productivity[1,2]

Patrick J. Mulholland,[3] J. W. Elwood, and A. V. Palumbo

Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830, USA

and R. J. Stevenson

Department of Biology, University of Louisville, Louisville, KY 40292, USA

Mulholland, P. J., J. W. Elwood, A. V. Palumbo, and R. J. Stevenson. 1986. Effect of stream acidification on periphyton composition, chlorophyll, and productivity. Can. J. Fish. Aquat. Sci. 43: 1846–1858.

We determined periphyton community composition, cell number and biovolume, chlorophyll *a* density, and areal and chlorophyll-specific primary productivity along a pH (4.5–6.4) and total monomeric aluminum (0.018–0.242 $mg \cdot L^{-1}$) gradient in high-elevation streams in the Great Smoky Mountains National Park (eastern Tennessee). The periphyton community was generally dominated by small chrysophytes and blue-green algae at sites with baseflow pH $\geq$ 5.5 and by diatoms and, to a lesser extent, green algae at sites with pH $\leq$ 5. Total cell biovolume, chlorophyll *a* density, and areal primary productivity were greatest at the most acidic sites. Chlorophyll-specific rates of primary production were not significantly different among sites, although the most acidic site was lowest. Experiments involving short-term manipulation of pH, inorganic carbon, PO_4, and aluminum concentrations resulted in some statistically significant changes in chlorophyll-specific productivity, indicating that inorganic carbon concentrations at sites with pH < 6 and increased aluminum concentrations at sites with pH $\leq$ 5 may limit productivity at times. Studies of phosphatase activity and PO_4 turnover indicated that the least acidic sites were the most phosphorus limited, perhaps the result of coprecipitation of aluminum and PO_4 as pH increased downstream from acidified reaches. However, the low cell biovolume, chlorophyll *a* density, and areal primary productivity at the second-order sites with pH $\geq$ 5.7, compared with the highly acidic second- and third-order sites (pH $\leq$ 5), appear to be the result of increased density of scraper/grazer macroinvertebrates at the higher pH sites. Low chlorophyll *a* density and areal primary productivity at the much larger fourth-order site (pH 6.4) may be the result of physical factors, such as increased scouring of the streambed, rather than chemical or biological factors.

Nous avons déterminé la composition de la communauté périphytonique, le nombre de cellules et le biovolume, la teneur en chlorophylle *a* et la productivité primaire surfacique et spécifique à la chlorophylle le long d'un gradient de pH (4,5 à 6,4) et de concentrations d'aluminium monomérique total (0,018 à 0,242 $mg \cdot L^{-1}$) dans des cours d'eau de haute altitude situés dans le parc national Great Smoky Mountains, dans l'est du Tennessee. La communauté périphytonique était généralement dominée par de petits chrysophytes et des algues bleu-vert aux endroits où le pH de l'écoulement de base était supérieur ou égal à 5,7 et par des diatomées et, en moindre quantité, des algues vertes aux endroits où le pH était inférieur ou égal à 5. La teneur en chlorophylle *a* et la productivité primaire surfacique étaient les plus élevées aux lieux les plus acides. Les taux de la production primaire spécifique à la chlorophylle ne différaient pas de façon significative entre les lieux, mais la plus faible valeur a été notée au lieu le plus acide. Des essais portant sur la modification à court terme du pH, des concentrations du carbone inorganique, du PO_4 et de l'aluminium se sont traduits par certaines modifications statistiquement significatives de la productivité spécifique à la chlorophylle; les concentrations de carbone inorganique aux sites à pH inférieur à 6 et l'augmentation des concentrations d'aluminium aux sites à pH inférieur ou égal à 5 peut parfois restreindre la productivité. Des études de l'activité de la phosphatase et du renouvellement du PO_4 ont montré que deux des sites les moins acides étaient ceux qui étaient le plus limités par le phosphore, peut-être à cause d'une co-précipitation de l'aluminium et du PO_4 à mesure que le pH augmentait en aval des zones acidifiées. La faible valeur de la teneur en chlorophylle *a* et de la productivité primaire surfacique aux sites d'ordre deux avec un pH $\geq$ 5,7 comparativement aux sites fortement acides (pH $\leq$ 5) d'ordres deux et trois semble cependant résulter d'une augmentation de la densité des macro-invertébrés racleurs et brouteurs aux sites plus acides. La faible teneur en chlorophylle *a* et la faible productivité primaire surfacique au site beaucoup plus important d'ordre quatre (pH 6,4) peut s'expliquer par des facteurs physiques, notamment un plus important raclage du lit du cours d'eau, plutôt que par des facteurs chimiques ou biologiques.

Received October 4, 1985
Accepted April 22, 1986
(J8472)

Reçu le 4 octobre 1985
Accepté le 22 avril 1986

[1]Research sponsored by the Electric Power Research Institute under contract RP2326-1 with the U.S. Department of Energy, under Contract No. DE-AC05-840R21400 with Martin Marietta Energy Systems, Inc.

[2]Publication No. 2735, Environmental Sciences Division, ORNL.

[3]Author to whom correspondence should be addressed.

Most studies of the effects of acidification on aquatic plants have focused on lake phytoplankton and have indicated reduced species richness, biomass, and productivity with acidification (Almer et al. 1974; Hendrey and Wright 1976; Kwiatkowski and Roff 1976; Yan 1979; Bleiwas et al. 1984). However, in a multiyear experimental acidification of a Canadian Shield lake, Schindler et al. (1985) found no decline in phytoplankton biomass and productivity despite a shift from a largely chrysophycean community to one dominated by chlorophycean, cyanophycean, and peridinean species. In the same experiment, Muller (1980) observed an increase in periphyton biomass with reduction in lake pH. In a recent study of benthic algal communities in poorly buffered New Hampshire lakes, Stevenson et al. (1985) reported that species composition, particularly among diatoms, varied with pH much more than did total algal biovolume.

The effect of acidification on stream periphyton may be different from that on lake phytoplankton as a result of (1) the close interaction between periphyton and sediments, (2) the large temporal and spatial variability in physical and chemical conditions in lotic ecosystems, and (3) the importance of flow in continuously renewing the water mass in lotic ecosystems. Comprehensive studies of the response of periphyton to stream acidification are few. Reduction in periphyton species richness has been reported for streams receiving acid mine drainage in a variety of locations (Bennett 1969; Whitton 1972; Lampkin and Sommerfeld 1982). However, acid mine drainage stress, particularly from toxic metals, is probably more severe than that from acidification due to atmospheric deposition of H_2SO_4 and HNO_3. In an experimental addition of sulfuric acid to artificial streams, reducing the pH to 4, Hendrey (1976) reported increased chlorophyll density, ash-free dry mass, and areal productivity of periphyton, although chlorophyll-specific productivity was reduced. Hendrey suggested that higher periphyton biomass resulted from reduced grazing by benthic invertebrates under acidic conditions. Increased levels of periphyton chlorophyll and ash-free dry mass were also observed in an experimentally acidified stream in the Hubbard Brook Experimental Forest, New Hampshire; however, the difference in chlorophyll content between acidified and non-acidified reaches was eliminated following a storm event (Hall et al. 1980).

We conducted a study of the effects of watershed acidification on the periphyton community in natural streams. Previous research involving experimental acid addition directly to stream channels (Hendrey 1976; Hall et al. 1980) documented effects resulting from within-channel pH change, but did not permit evaluation of the effects of materials mobilized from soils as a result of watershed acidification. We measured structural and functional properties of periphyton communities over a pH and dissolved-aluminum gradient in low-alkalinity, forested, mountain streams in the Great Smoky Mountains National Park in eastern Tennessee. In addition, we conducted experimental studies of phosphorus limitation, inorganic carbon limitation, and aluminum inhibition of periphyton at several stream sites. Levels of sulfate (3.6−4.5 $mg \cdot L^{-1}$) and trace metals at our most acidic sites are similar to those found in streams draining poorly buffered watersheds impacted by acid precipitation in other regions (Driscoll 1980; Johnson et al. 1981). However, we are uncertain whether anthropogenic acid deposition and differential buffering in subcatchments or internal watershed sources of acidity (e.g. pyrite oxidation, nitrification) are primarily responsible for different levels of acidity in Walker Camp Prong.

Description of Study Site

Walker Camp Prong is a tributary of the West Prong Little Pigeon River and drains high-elevation areas near Newfound Gap in the Great Smoky Mountains National Park (GSMNP) (eastern Tennessee). The climate is humid microthermal with an average annual precipitation of approximately 220 cm (Shanks 1954). The area consists of Precambrian to Cambrian metasedimentary rock, mostly quartzite and phyllite, which is relatively resistant to weathering and therefore provides little neutralization capacity (King et al. 1968). Outcroppings of a pyritic carbonaceous phyllite, the Anakeesta Formation, occur at higher elevations (King et al. 1968). Soils are poorly developed, high in organic matter content, and acidic (Herrman and Baron 1980). Vegetation is dominated by mature stands of red spruce and Fraser fir at high elevation, with beech and hemlock becoming important at lower elevations and in riparian zones. Rhododendron is abundant, forming a dense subcanopy at high elevations and near stream channels at all elevations. The topography is steep with streams flowing in deeply incised valleys.

Walker Camp Prong is a rocky, high-gradient mountain stream. Our study sites included two second-order reaches near their confluence (WP4 and WP5, elevation 1440 m), one third-order reach (WP3), and one fourth-order reach (WP1, elevation 1130 m) of Walker Camp Prong (Fig. 1). Additional study sites included two second-order, high-gradient tributaries of Walker Camp Prong, Trout Branch (TB) and Cole Creek (CO). Baseflow water chemistry data are given in Table 1. The sites are all low in alkalinity and ionic strength, but vary in pH from 4.5 to 6.4 and in total monomeric aluminum from 0.020 to 0.272 $mg \cdot L^{-1}$ at baseflow. The low pH values (<6) have been attributed to pyrite oxidation at exposed outcrops of the Anakeesta Formation within subcatchments of Walker Camp Prong (Huckabee et al. 1975), although the contribution of other internal (nitrification, organic acids) and external (acid precipitation) sources of acidity may also be important. Trace metal data from the most acidic sites (WP3, WP4, and TB) at baseflow indicate that only total monomeric aluminum, manganese, and to a lesser extent iron are elevated above levels at the less acidic sites (Table 1). A few samples collected during stormflow indicated that pH declined and aluminum increased at all stations compared with baseflow, with the greatest changes occurring at the highest order station (WP1) (pH's as low as 4.9) as the acidity gradient expanded downstream. The smallest reduction in stormflow pH (0.1−0.3 unit) occurred at the most acidic site (WP4). Depressions in stream pH and increases in aluminum during storms have been recorded for several streams in the GSMNP (Herrman and Baron 1980; Jones et al. 1983).

Stream study sites were chosen to minimize differences in physical conditions and yet provide a gradient in pH and aluminum concentrations. All sites had incomplete canopy cover, allowing direct sunlight to reach the stream for periods each day during the growing season. All sites were in areas of relatively high water velocity (>30 $cm \cdot s^{-1}$) to minimize diffusion limitations of nutrient uptake by periphyton. The second- and third-order sites (WP4, WP5, TB, CO, and WP3) were not greatly different in drainage basin size, stream width,

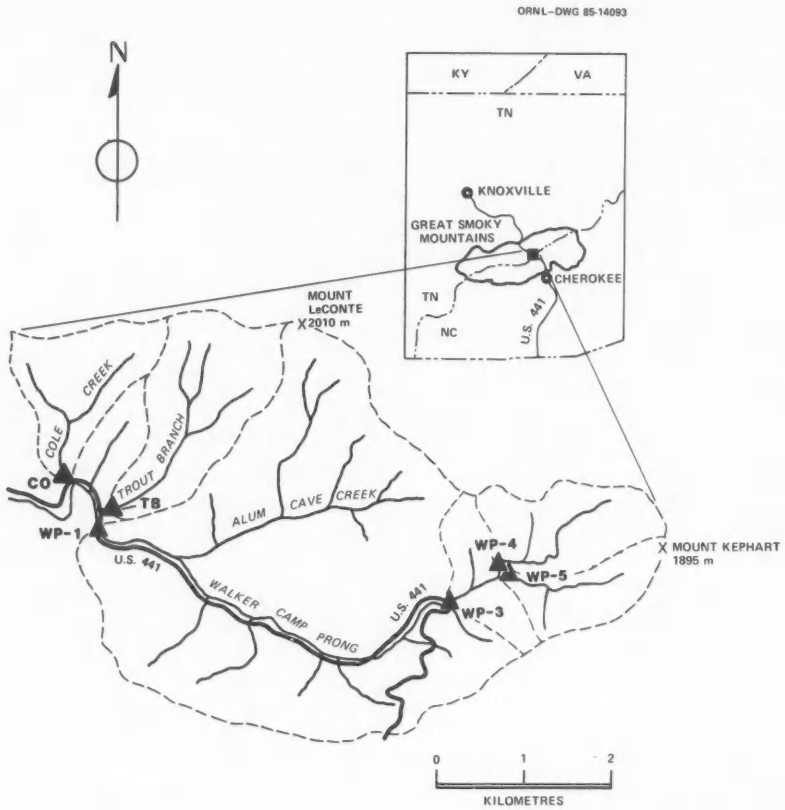


FIG. 1. Walker Camp Prong with sampling sites and topographic boundaries indicated.

and gradient (Table 1). However, the fourth-order site (WP1) was considerably wider and drained a much larger area than the other sites and therefore had somewhat longer periods of direct sunlight and much greater flow than did the other sites.

Methods

Periphyton community composition, cell number, and cell biovolume were measured in mid-November 1984 on small, flat rocks (upper surface area of 15–50 cm^2) and in May 1985 on clay tiles (43 cm^2) placed in riffle areas at each site in Walker Camp Prong. Clay tiles were used as surrogates for natural rock substrata to reduce variability in periphyton abundance resulting from irregularity of rock surfaces and the tendency of small rocks to upturn or roll. To ensure establishment of a representative periphyton community, the tiles were glued to bricks staked to the streambed at least 10 wk prior to sampling. Other studies have demonstrated that clay tiles develop a periphyton community very similar in composition and biomass to that on natural substrata in streams, and that a colonization period of 10 wk is sufficient to develop this community (Tuchman and Stevenson 1980; Lamberti and Resh 1985).

To determine the response of the periphyton community to environmental changes associated with acidification, we conducted a reciprocal transplant study of periphyton communities between an acidified (TB) and a nonacidified site (CO) in July 1985. After an initial 10-wk colonization period, periphyton-colonized tiles (glued to bricks) at TB and CO were carefully removed from the stream, placed in buckets containing stream water, transported to the other site, and placed in the same locations from which tiles were removed. The transplant was completed within 30 min. Measurements of community composition, cell number, and cell biovolume were made initially and at 9 and 21 d following the transplant on transplanted tiles and tiles which were left intact at each site.

The rocks or tiles used for periphyton enumeration were immersed in Lugol's solution immediately after collection and returned to the laboratory. Periphyton were removed from rocks or tiles by brushing the substratum surface, rinsing the brush and surface with distilled water, and collecting the slurry in 250-mL beakers. This procedure was continued until the rinse water was no longer turbid. Syrup mounts of the algal suspensions from each substratum were made for total viable algal cell counts and biovolume measurements according to procedures described in Stevenson (1984) and Stevenson et al. (1985). Burn mounts of the algal suspensions were made to determine diatom taxonomy.

Periphyton chlorophyll *a* and phaeophytin densities were determined during July and October 1984 on small, flat rocks collected from riffle areas and again in May 1985 on clay tiles at the Walker Camp Prong sites. The rocks or tiles were placed

TABLE 1. Physical and chemical data from four sites on Walker Camp Prong (WP) and two of its tributaries (TB and CO). Chemical values are averages of monthly samples during baseflow periods. All values are $mg \cdot L^{-1}$ unless indicated otherwise.

Parameter	WP1	WP3	WP4	WP5	TB	CO
Drainage area (km^2)	17.8	3.8	1.8	1.2	2.8	1.8
Stream order	4	3	2	2	2	2
Stream gradient (%)	6	10	8	8	20	14
Width (m)	12	6	5	4	4	3
Conductance ($\mu S \cdot cm^{-1}$)	23.7	24.8	30.8	19.4	22.9	19.0
Acid neutralizing capacity ($\mu eq \cdot L^{-1}$)	28.5	−16.6	−30.8	−0.1	−15.3	19.9
Median pH	6.4	4.8	4.5	5.7	5.0	6.4
F	0.021	0.020	0.017	0.016	0.012	0.010
Cl	0.58	0.47	0.50	0.43	0.60	0.62
SO_4	4.50	3.59	3.95	2.82	4.25	1.97
NO_3	3.01	4.06	4.02	4.56	3.35	3.78
NH_4	0.10	0.09	0.13	0.06	<0.01	<0.01
Na	0.56	0.42	0.34	0.50	0.40	0.78
K	0.23	0.20	0.20	0.19	0.25	0.37
Ca	2.21	1.36	1.21	1.68	1.23	1.52
Mg	0.76	0.55	0.50	0.60	0.70	0.64
Mn	0.006	0.043	0.055	0.003	0.041	0.001
Fe	0.010	0.018	0.065	0.003	0.004	0.007
Si	1.59	1.25	1.08	1.41	1.31	2.11
Al (total monomeric)[a]	0.018	0.145	0.242	0.027	0.133	0.018
Al (organic monomeric)[a]	0.011	0.029	0.050	0.009	0.012	0.015
Soluble reactive phosphorus ($\mu g \cdot L^{-1}$)	0.9	0.8	1.3	0.7	0.5	1.2
Total soluble phosphorus ($\mu g \cdot L^{-1}$)	2.2	2.9	3.3	1.9	1.5	3.1
Total inorganic carbon	0.76	0.36	0.37	0.42	0.36	0.69
Dissolved organic carbon	0.8	1.3	2.1	0.5	0.7	1.1

[a]Fractionation technique of Driscoll (1984).

in darkened jars containing 50 mL of 100% methanol in July 1984 or dimethyl sulfoxide (DMSO) thereafter, and pigments were extracted overnight at room temperature. Chlorophyll *a* and phaeophytin were measured according to procedures outlined by Marker (1972) and Holm-Hansen and Riemann (1978) for methanol and by Shoaf and Lium (1976) and Burnison (1980) for DMSO. For methanol extraction, 5-mL subsamples were removed from the jars and the optical absorbance read at 665 and 750 nm. The subsamples were then acidified with 150 μL of 0.1 *M* HCl and, after 3 min, neutralized with 50 mg of $MgCO_3$. After 10 min, the neutralized extract was filtered and the absorbance reread at 665 and 750 nm. The DMSO technique involved a 1:1 dilution of the extract with 90% acetone and measurement of optical absorbance at 665 and 750 nm before and after acidification with HCl. The extraction efficiencies of the methanol and DMSO methods were compared by collecting 20 rocks at random from a single site and subjecting half to methanol extraction and the other half to DMSO extraction. The 8% greater average chlorophyll *a* density measured using the methanol method was not significant ($P < 0.05$, Palumbo et al. 1987).

Periphyton productivity was measured using closed 2000-mL recirculating chambers as described by Rogers et al. (1978). The chambers were identically constructed and operated to eliminate differences in recirculation rate during incubations. Recirculation rate was observed to be very rapid, with injected dye completely mixed within 5 s. Several (three to five) replicate cobble-sized rocks or clay tiles at each site were carefully removed from the stream, immediately transferred to a chamber containing streamwater from that site, and transported to a central location (near WP3) and submerged to eliminate any differences between sites in light and temperature during the incubation period. Periphyton substrata were collected only from areas of high stream velocity ($>30 cm \cdot s^{-1}$) at each site to minimize inclusion of settled and loosely attached material. Generally, one chamber was used to incubate all replicate substrata from a given site. On one occasion when duplicate chambers were run for one site, mean rates of chlorophyll-specific productivity on substrata in each chamber were within 8% and were not significantly different ($P < 0.01$). To further assess the variability in production measurements due to differences among chambers, we conducted an experiment using two chambers at each of two sites. Using this design, we determined that all of the variability in the measured rates of chlorophyll-specific productivity was a result of differences between sites and random error (ANOVA, nested design, SAS Institute 1982).

Rates of periphyton productivity were measured using the ^{14}C-uptake method (Steemann Nielsen 1952). Approximately 20 μCi of high specific activity [^{14}C]HCO_3 (2.11 $GBq \cdot mmol^{-1}$; Amersham Corp.) was injected through a septum into each submerged chamber containing three to five rocks or tiles and streamwater from each site. Total addition of inorganic carbon (including carrier) was 0.07 mg and amounted to a small increase in streamwater inorganic carbon concentrations (≤15% of ambient). After an incubation period of 1−2 h, the submerged chambers were removed from the stream, and the substrata were taken out of each chamber and rinsed with the

acidic streamwater (pH 4.7−4.9) to remove nonincorporated ^{14}C. The substrata were then each placed in a wide-mouth jar containing 30 mL of DMSO for extraction of ^{14}C-labeled photosynthate (Filbin and Hough 1984) and chlorophyll *a*. The samples were extracted at room temperature overnight, and after removal of subsamples for chlorophyll *a* measurement, were heated to 65°C and agitated periodically for 6 h to further extract ^{14}C-labeled photosynthate. Subsamples (0.1 mL) of the extract were then removed and assayed for ^{14}C by liquid scintillation counting using standard additions to compute counting efficiency. To determine residual ^{14}C-labeled photosynthate not extracted by DMSO, several rocks and tiles were subsequently placed in a sulfuric acid − dichromate solution in a sealed combustion chamber and heated for 1 h at 100°C (suggested by Strickland and Parsons (1972) for oxidation of particulate organic matter). Evolved $^{14}CO_2$ was absorbed in 2 *M* KOH and assayed by liquid scintillation counting. The DMSO procedure was found to extract 76% (standard error 4%, $n = 14$) of the ^{14}C taken up by periphyton on rock surfaces and 92% (standard error 3%, $n = 12$) of that taken up by periphyton on tile surfaces (Palumbo et al. 1987). Values of total ^{14}C uptake by periphyton as measured by DMSO extraction were therefore increased by factors of 1.32 for rocks and 1.09 for tiles to account for the incomplete extraction.

Abiotic sorption of ^{14}C, measured using dry rocks collected from portions of the streambed wetted only during large storms and incubated with the periphyton-colonized substrata, was negligible. Dark uptake of ^{14}C by periphyton, determined using darkened chambers on one occasion, was <2% of uptake in the light. Measurement of ^{14}C activity in chambers after acidification and sparging with nitrogen gas indicated that <5% of total ^{14}C uptake was released back to the water in organic form during the incubation.

Photosynthetically active radiation (PAR) was measured at the water surface during each incubation using a LiCor (model 188B) integrating quantum meter.

Streamwater pH was measured using a Ross combination electrode (Orion Research, Inc.). Water samples for total inorganic carbon (TIC), soluble reactive phosphorus (SRP), and total soluble phosphorus (TSP) concentrations were taken from the stream at each site at the beginning of each incubation. TIC samples were collected in glass bottles or polyethylene bags, placed on ice and returned to the laboratory, and total TIC measured in 10-mL subsamples using an OI model 700 total carbon analyzer in the TIC analysis mode (infrared absorption of CO_2 gas following acid addition and N_2 stripping). Samples (100 mL) for SRP and TSP analyses were filtered (0.4-μm Nuclepore) in the field. SRP was analyzed according to the procedure of Murphy and Riley (1962), and TSP was determined after persulfate oxidation (American Public Health Association 1985).

To determine the influence of water chemistry changes associated with acidification on periphyton productivity, we experimentally altered several water chemistry parameters independently during short-term (2 h) incubations of periphyton and streamwater from several sites in October and November 1984 and in May 1985. Immediately prior to ^{14}C inoculation, water chemistry in the recirculating chambers was modified by one of the following additions: (1) H_2SO_4 to reduce pH in water from the least acidic site (WP1), (2) $NaHCO_3$ and H_2SO_4 to increase TIC concentrations while maintaining ambient pH, (3) sodium citrate to complex free trace metals in solution, (4) $AlCl_3$ to increase monomeric aluminum concentrations, or (5) Na_2HPO_4 to increase PO_4 concentrations. In addition, on one occasion, TIC was reduced at a highly acidic site (WP3) by shaking streamwater in partially filled bottles thereby degassing CO_2 from normally supersaturated concentrations (approximately 1.5−2 times saturation level).

To determine the relative degree of phosphorus limitation between sites, phosphatase assays were conducted in September and November 1985. Small, flat rocks (approximately 25 cm^2) were randomly collected from high-velocity areas at each site and immersed in 60 mL of streamwater in jars. After addition of an organic phosphorus substrate (*p*-nitrophenyl phosphate, Sigma 104) to each jar (yielding a concentration of 2.5 m*M* which was determined in a separate study to saturate the natural periphyton enzyme system), the jars were incubated for 0.5 h at one site to eliminate temperature differences. Immediately following incubation, the streamwater in each jar was filtered (0.45 μm) and returned to the laboratory on ice. The pH of the filtered water was then increased (pH > 10) to promote maximum color development of nitrophenyl (NP) produced as a result of phosphatase activity and the absorbance read at 410 nm against distilled water. Levels of ATP on each rock were also measured to normalize phosphatase activity for the amount of microbial biomass present. Following the incubation, each rock was rinsed in streamwater and immersed in 1 *M* H_3PO_4 overnight for ATP extraction and measurement according to the method of Karl and Craven (1980).

To further assess differences in phosphorus dynamics between a highly acidic (WP4) and slightly acidic (WP5) site, relative rates of PO_4 turnover in the water resulting from uptake by the periphyton community were measured in December 1985 using a radiotracer. After >6 mo in the stream, two tiles were carefully removed from each site, placed in jars containing streamwater from that site, placed on ice, and returned to the laboratory. In the laboratory (within 3 h of collection in the field) the tiles from each site were placed in glass containers with 1 L of streamwater, circulated by magnetic stirrers, maintained in darkness at 10°C, and inoculated with 10 μCi of carrier-free $^{33}PO_4$. Measurements of ^{33}P activity in filtrate (dissolved) and retained on 0.1-μm filters (Nuclepore) were made over the next 20 h. Following the incubation the tiles were removed from the containers and subjected to sequential extraction in 1 *M* H_3PO_4 (24 h) and a 0.3 *M* NaOH − 1% sodium dodecylsulfate − 25 m*M* EDTA solution (24 h) to partition ^{33}P taken up by the periphyton community into acid-labile (e.g. ATP) and nonlabile (e.g. DNA) pools (see Findlay et al. 1984).

To determine, at least indirectly, the effect of grazing, stream macroinvertebrate abundance was measured in April 1985 using a modified Hess sampler (0.1 m^2). Samples were collected from five riffle areas at each site by vigorously agitating the streambed to a depth of approximately 15 cm or to bedrock. Organisms were removed from the Hess sampler, fixed with formaldehyde, and identified and counted in the laboratory using a dissecting microscope. Macroinvertebrates were grouped as scraper/grazers according to the functional classification given by Merritt and Cummins (1984).

Results and Discussion

Community Composition and Cell Abundance

The periphyton community differed markedly among sites in Walker Camp Prong. In November 1984, the most acidic sites (WP4 and WP3) were dominated by diatoms (particularly

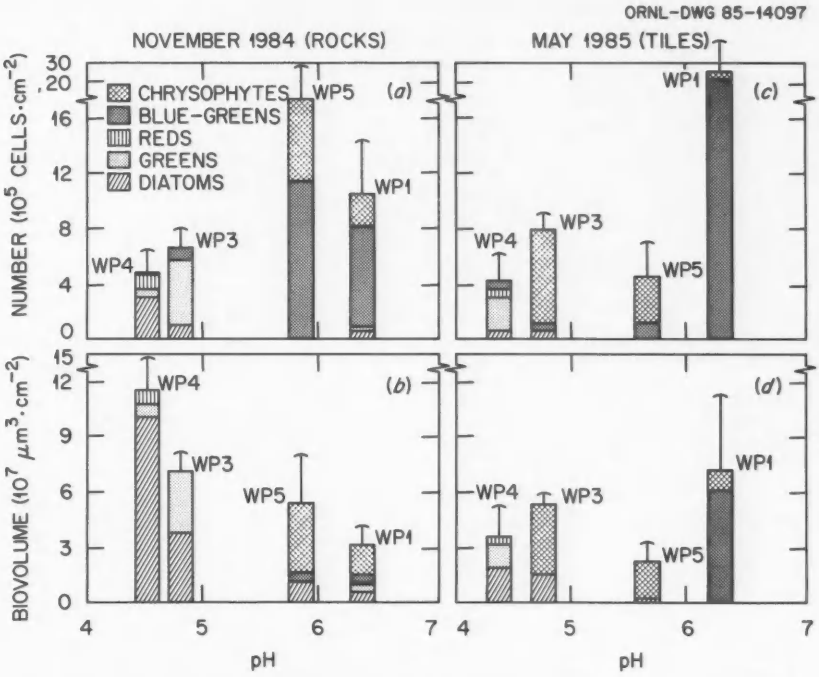


FIG. 2. Periphyton cell number, biovolume, and species composition along a gradient in pH in Walker Camp Prong on two dates. Vertical bars indicate one standard error ($n = 3$).

Eunotia lunaris and *E. arcus*) and small unidentified green algae (Fig. 2a, 2b). At the less acidic sites (WP5 and WP1), blue-green algae (particularly *Lyngbya penicillata, Anacystis marina, Coccochloris stagnina,* and small chameosiphonalean algae) were very abundant, but diatoms (particularly *E. lunaris* and *E. pectinalis*) and unidentified chrysophytes dominated total biovolume. In May 1985, blue-green algae (primarily *Entophysalis* sp. and small chameosiphonalean algae) dominated both cell density and biovolume at WP1, but were less abundant at WP5, compared with November 1984 (Fig. 2c, 2d). Also in May, one of the highly acidic sites (WP3) was dominated by unidentified chrysophytes with no evidence of the green algae which were an important component of the community in November. At the most acidic site (WP4) in May, the diatom *E. rhomboidea* replaced *E. arcus* as a co-dominant with *E. lunaris*. Also at WP4, in both November and May, a red alga from the genus *Rhodochorton* was a prominent component of the periphyton community.

Despite a dominance by diatoms at the most acidic sites, the mean number of diatom taxa observed during cell counts was lowest at the most acidic site and increased with increasing pH (9.3, 11.3, 13.7, and 18.0 at WP4, WP3, WP5, and WP1, respectively). However, our data do not necessarily indicate a simplification of the entire periphyton community with reduced pH because other groups of algae, particularly red and green algae, increased in importance at the highly acidic sites. The lack of importance of blue-green algae at the highly acidic sites (WP4 and WP3) supports the hypothesis of Brock (1973) that procaryotic algae are not present at pH < 5 because their chlorophyll is not adequately protected from external acidic conditions.

In November, cell numbers were lower but total cell biovolume was greater at the most acidic sites (WP4 and WP3) compared with the less acidic sites (WP5 and WP1) (Fig. 2a, 2b). The relatively high cell numbers, but lower cell biovolumes, at the less acidic sites were primarily the result of dominance by small blue-green algae at these sites. In May, however, this pattern was not observed (Fig. 2c, 2d). Comparing the two second-order stream sites only (WP4 and WP5), cell numbers were lower and cell biovolume greater at the more acidic site on both sampling dates, although the differences in May were not significant ($P < 0.05$).

The patterns in periphyton community composition, cell number, and cell biovolume with pH observed in the studies of Walker Camp Prong sites were also observed in the comparison of highly acidic (TB) and nonacidified (CO) tributaries of Walker Camp Prong (Fig. 3). Periphyton cell numbers were greater in CO, whereas cell biovolumes were greater in TB throughout July 1985. Blue-green algae (particularly *Anacystis marina, Coccochloris peniocystis, Entophysalis* sp., and small chameosiphonalean algae) dominated the cell biovolume at CO, although diatoms (particularly *Eunotia pectinalis* and *E. rhomboidea*) and small unidentified chrysophytes were also important components. At TB, diatoms (particularly *Eunotia incisa* and *E. pectinalis*) dominated cell biovolume.

When periphyton-colonized tiles were transplanted between CO and TB, community composition and abundance changed relatively slowly in response to the change in environmental conditions (Fig. 3). Nine days after the transplant, cell biovolume on CO tiles moved to TB were substantially increased and biovolume on TB tiles moved to CO were substantially reduced relative to tiles which were not moved. However, even

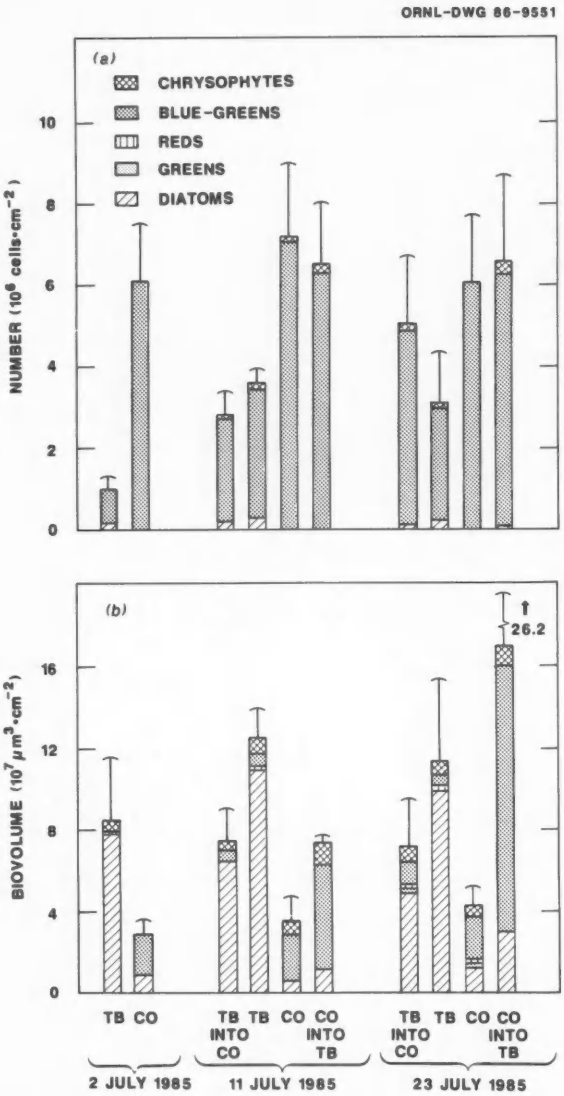


FIG. 3. Periphyton cell number, biovolume, and species composition in Walker Camp Prong tributaries TB (pH 4.9–5.1) and CO (pH 6.2–6.5) on three dates during July 1985. Also shown are cell number, biovolume, and species composition of periphyton communities which were transplanted between these sites on 2 July and measured on 11 July and 23 July, 9 and 21 d after the transplant. Vertical bars indicate one standard error (n = 3).

3 wk after the transplant, cell biovolume on tiles transplanted from CO to TB were still dominated by blue-green algae and the TB to CO transplants were still dominated by diatoms. Evidently, shifts in species composition with changing environmental conditions related to acidification occur more slowly than changes in total cell biovolume. The environmental conditions at the acidic TB site (pH 4.9–5.1) were not acutely toxic to periphyton from a nonacidified site (CO, pH 6.2–6.5).

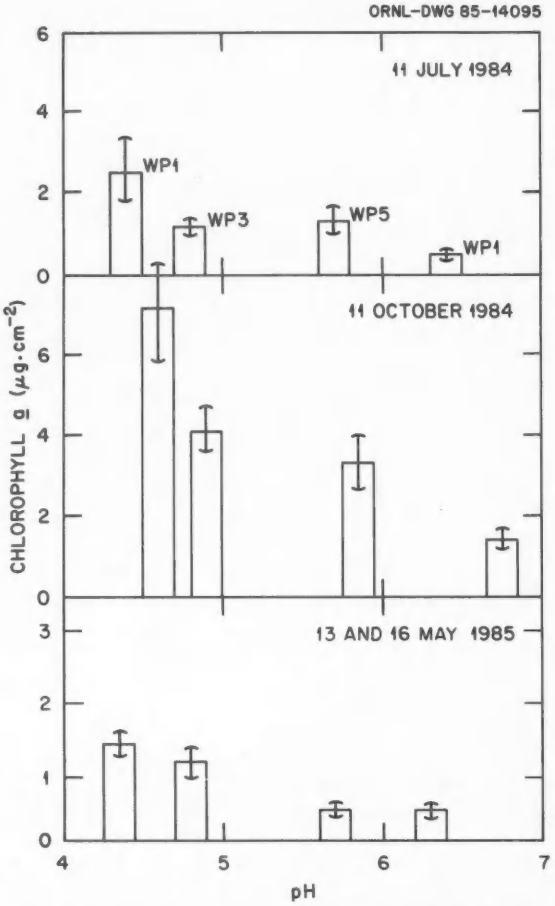


FIG. 4. Periphyton chlorophyll a densities at four sites in Walker Camp Prong measured on three dates and plotted as a function of streamwater pH. Vertical bars indicate one standard error (n = 5).

Chlorophyll *a* Density and Productivity

Periphyton chlorophyll a densities were inversely related to pH (Fig. 4). Chlorophyll a densities were greater at Walker Camp Prong sites in October 1984 following a 6- to 8-wk period of low precipitation and stable streamflow which permitted a greater accumulation of periphyton than in July 1984 or May 1985. Increased frequency of large storms which scour the streambed may partially mask the relationship between pH and chlorophyll a density. Chlorophyll a densities at TB and CO also followed the pattern of higher density at low pH. Average chlorophyll a density at TB for four sampling periods in July and August 1985 was 1.7 μg·cm^{-2} (SD = 0.4), whereas average chlorophyll a density at CO for the same dates was 1.2 μg·cm^{-2} (SD = 0.1). Phaeophytin densities were at all times and at all sites very low (usually <5% of chlorophyll a density).

Areal primary productivity was also inversely related to pH for the Walker Camp Prong sites (Fig. 5). Similarly, average areal primary productivity for four dates in July–August 1985 in highly acidic TB (3.6 mg C·m^{-2}·h^{-1}, SD = 0.2) was greater

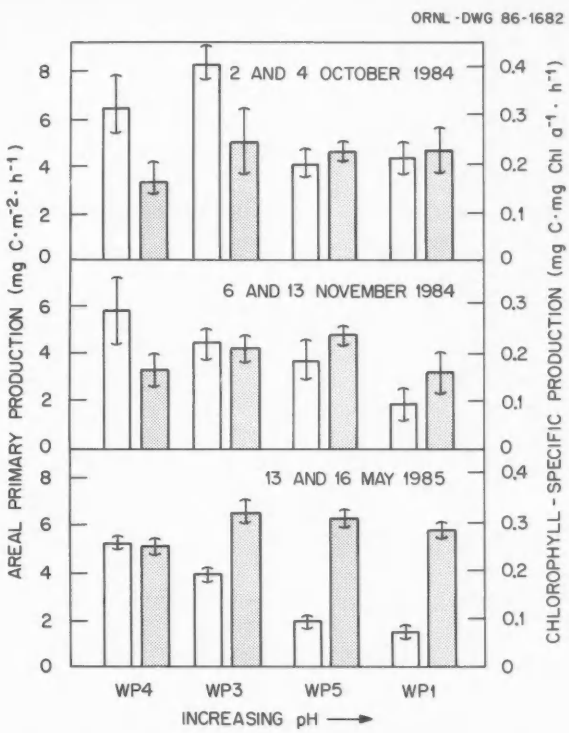


FIG. 5. Areal primary productivity (open) and chlorophyll-specific productivity (shaded) at four sites in Walker Camp Prong. Although productivity at different sites was measured on different dates (WP4 and WP5 on 2 October, 13 November, and 13 May; WP1 and WP3 on 4 October, 6 November, and 16 May), PAR was probably above saturation levels on all dates and water temperatures were similar during each pair of studies (323 and 284 $\mu E \cdot m^{-2} \cdot s^{-1}$ and 9°C in October; 469 and 640 $\mu E \cdot m^{-2} \cdot s^{-1}$ and 6 and 4°C in November; 246 and >900 $\mu E \cdot m^{-2} \cdot s^{-1}$ and 12°C in May).

than that for the same dates in CO (2.5 mg $C \cdot m^{-2} \cdot h^{-1}$, SD = 0.6). Areal primary productivities in Walker Camp Prong were 2–5 times greater than values reported for some other forested streams in Tennessee (Elwood and Nelson 1972), Kentucky (Minshall 1967), and Virginia (Hornick et al. 1981) and for two other streams, Camel Hump and Husky Branch, in the GSMNP (Keithan and Lowe 1985). Chlorophyll *a* densities in Walker Camp Prong were about 10 times greater than those in Camel Hump and Husky Branch. The greater chlorophyll *a* densities and areal primary productivity of Walker Camp Prong compared with the two other GSMNP streams may be due to greater PAR in Walker Camp Prong (> 250 $\mu E \cdot m^{-2} \cdot s^{-1}$). PAR measurements in Camel Hump and Husky Branch were generally <50 $\mu E \cdot m^{-2} \cdot s^{-1}$ during the growing season (Keithan and Lowe 1985). Turner et al. (1983) demonstrated that the relationship between ^{14}C uptake by lake periphyton and PAR can be described by a Michaelis–Menten type relationship. They computed half-saturation constants of from 10 to 45 $\mu E \cdot m^{-2} \cdot s^{-1}$, indicating strong light limitation of ^{14}C uptake at PAR < 50 $\mu E \cdot m^{-2} \cdot s^{-1}$. McIntire and Phinney (1965) have shown that stream periphyton production was saturated at illumination intensities above approximately 200 $\mu E \cdot m^{-2} \cdot s^{-1}$.

Our results indicating greater chlorophyll *a* density and areal primary productivity at the more acidic stream sites in Walker Camp Prong are similar to those of Hendrey (1976) for an experimentally acidified artificial stream in Norway. Our results indicating greater chlorophyll *a* density at the more acidic stream sites are also consistent with those of Hall et al. (1980) for an experimentally acidified stream in New Hampshire. However, our results conflict with those of Winterbourn et al. (1985) who reported reduced chlorophyll *a* densities in English streams with pH < 5.5 compared with those with pH > 6.

We found no significant differences in chlorophyll-specific productivity among the different sites for all studies conducted (Fig. 5). However, chlorophyll-specific productivity at the most acidic site (WP4) was consistently low compared with the other sites. Chlorophyll-specific productivity in May 1985 was significantly greater at all sites than values measured in November 1984 ($P < 0.05$), a difference which could be due to higher water temperature in May (12°C) than in November (4–6°C). Average PAR appears to have been near or at the saturation level for photosynthesis during all studies. Despite variation from 246 (WP4 and WP5, 13 May 1985) to >1000 $\mu E \cdot m^{-2} \cdot s^{-1}$ (WP1 and WP3, 16 May 1985), there was little difference in chlorophyll-specific productivity between sites.

Water Chemistry Factors Controlling Periphyton Productivity

Acidification could potentially reduce periphyton productivity in streams as a result of several water chemistry changes: (1) inhibition by high hydrogen ion concentrations, (2) inorganic carbon limitation resulting from reduced TIC supplies in streamwater at low pH, (3) inhibition by elevated trace metal concentrations, particularly inorganic monomeric aluminum, and (4) increased phosphorus limitation resulting from adsorption and/or co-precipitation of PO_4^{3-} with aluminum (mobilized from watershed soils and precipitated as aluminum trihydroxide) as pH increases (Hendrey et al. 1980). Our data on chlorophyll-specific productivity along a pH gradient in Walker Camp Prong suggest that there may be a small adverse effect on the efficiency of carbon uptake by periphyton at the most acidic site (WP4) (Fig. 5). However, differences in species composition between sites, which could result in differences in chlorophyll content per unit biomass, make site-to-site comparisons of photosynthetic efficiency problematic. In addition, greater periphyton biomass at WP4 (inferred from greater chlorophyll *a* density) could result in greater self-shading or diffusion resistance and consequently reduced chlorophyll-specific productivity at this site. Therefore, to determine the factors controlling chlorophyll-specific productivity at each site, a more direct assessment was necessary. To this end, we conducted several short-term experiments in the field involving incubations of periphyton and water chemistry manipulations.

Reduction in pH at WP1 from ambient values of 6.3–6.4 to 4.4–4.5 resulted in no significant change in chlorophyll-specific productivity (Table 2). Short-term (2 h) pH reduction to ≤4.5 did not inhibit photosynthesis of a periphyton community which normally experiences a pH > 6. These data indicate that the elimination of blue-green algae as an important component of the periphyton community at sites with pH < 5 (Fig. 2) is either the result of a more slowly developing inhibition of photosynthesis with increased hydrogen ion concentration or of changes in other environmental factors with acidification.

Concentrations of TIC were low at the sites with pH < 6 (Table 1) and appear at times to limit periphyton productivity.

TABLE 2. Effect of short-term (2 h) reduction of pH (addition of H_2SO_4) on chlorophyll-specific productivity (mg C·mg Chl a^{-1}·h^{-1}) of periphyton at WP1. Water temperature was 6 and 11.5°C and average PAR was 469 and >800 $\mu E \cdot m^{-2} \cdot s^{-1}$ in November and May, respectively. Differences between control and treatment productivity on each date were not significant at the $P < 0.05$ level. Standard error and number of replicates are given in parentheses.

	Control		Treatment	
Date	pH	Productivity	pH	Productivity
6 November 1984	6.4	0.145 (0.037, 5)	4.5	0.121 (0.012, 5)
16 May 1985	6.3	0.277 (0.022, 5)	4.4	0.255 (0.020, 5)

TABLE 3. Effect of short-term (2 h) alteration of TIC ($mg \cdot L^{-1}$) on chlorophyll-specific productivity (mg C·mg Chl a^{-1}·h^{-1}) at several sites. Water temperature and average PAR for 6 November and 16 May are the same as those given in Table 2. Water temperature and average PAR for 13 November were 4°C and 640 $\mu E \cdot m^{-2} \cdot s^{-1}$, respectively. Standard error and number of replicates are given in parentheses. An asterisk indicates that the treatment was significantly different from the control ($P < 0.05$).

		Control		Treatment	
Date	Site	DIC	Productivity	DIC	Productivity
6 November 1984	WP3	0.35	0.199 (0.031, 5)	0.84	0.157 (0.013, 5)
6 November 1984	WP3	0.35	0.199 (0.031, 5)	0.27	0.081 (0.005, 5)*
13 November 1984	WP4	0.38	0.157 (0.039, 5)	0.88	0.141 (0.017, 5)
13 November 1984	WP5	0.43	0.232 (0.018, 4)	0.94	0.471 (0.143, 5)*
16 May 1985	WP3	0.25	0.347 (0.017, 5)	1.06	0.793 (0.084, 5)*
16 May 1985	WP1	0.57	0.277 (0.022, 5)	1.21	0.393 (0.061, 5)

TABLE 4. Effect of short-term (2 h) additions of citrate and aluminum (as $AlCl_3$) on chlorophyll-specific productivity (mg C·mg Chl a^{-1}·h^{-1}) of periphyton at WP3 and WP4. Water temperatures were 11, 11.5, 6, and 4°C for the October, May, and two November experiments, respectively. The corresponding average PAR during these experiments were 486, >900, 469, and 640 $\mu E \cdot m^{-2} \cdot s^{-1}$, respectively. Standard error and number of replicates are given in parentheses. An asterisk indicates that the treatment was significantly different from the control ($P < 0.05$).

		Control		Treatment	
Date	Station	Al ($mg \cdot L^{-1}$)	Productivity	Addition	Productivity
16 October 1984	WP3	0.109	0.268 (0.046, 4)	Citrate (100 μM)	0.272 (0.038, 4)
16 May 1985	WP3	0.154	0.347 (0.019, 4)	Citrate (10 μM)	0.494 (0.094, 4)
6 November 1984	WP3	0.164	0.199 (0.031, 5)	Al (0.25 $mg \cdot L^{-1}$)	0.111 (0.039, 5)*
13 November 1984	WP4	0.336	0.157 (0.039, 5)	Al (0.25 $mg \cdot L^{-1}$)	0.140 (0.018, 4)

At the highly acidic sites (WP3 and WP4), results of short-term experiments involving manipulation of TIC concentrations alone indicated that inorganic carbon availability limited chlorophyll-specific productivity when TIC concentration dropped below about 0.3 $mg \cdot L^{-1}$ (Table 3). However, our data also indicated that inorganic carbon may limit productivity at somewhat greater TIC concentrations at the less acidic sites, particularly WP5. In a study of inorganic carbon limited algal growth, Goldman et al. (1974) measured Michaelis–Menten kinetics half-saturation constants of 0.14 and 0.30 mg $C \cdot L^{-1}$ for two species of green algae. Concentrations of TIC at our most acidic sites (WP4 and WP3) are, at times, similar to or less than the latter half-saturation constant. Further, a reduction of ambient TIC concentrations of up to 33% (from 0.35 to 0.24 $mg \cdot L^{-1}$) during the day was measured at WP3 on one date during spring.

Results from our short-term experiments concerning the effect of aluminum on productivity at the highly acidic sites were somewhat ambiguous. Addition of citrate to complex metals in solution and reduce their potential toxicity to biota resulted in no significant changes in chlorophyll-specific productivity at WP3 on two occasions, although in May 1985, large variability in the treatment values prevented a large increase in productivity with citrate addition from being statistically significant (Table 4). Aluminum additions of 0.25 $mg \cdot L^{-1}$ resulted in a significant decrease in chlorophyll-specific productivity at WP3 but no change at WP4 in November 1984. Perhaps the periphyton community at WP4 had adapted to the relatively high ambient aluminum concentrations at that site (Table 1). The experimental aluminum increase of 0.25 $mg \cdot L^{-1}$ represents a much greater proportional increase at WP3 and consequently may have had a greater effect on the periphyton community. Nalewajko and Paul (1985) have reported that phytoplankton photosynthesis was depressed when aluminum

TABLE 5. Effect of short-term PO_4 addition (10 $\mu g\ P \cdot L^{-1}$) on chlorophyll-specific productivity (mg C · mg Chl $a^{-1} \cdot h^{-1}$) of periphyton at WP1 and WP3. Conditions on 4 October 1984 were $T = 9°C$ and PAR = 284 $\mu E \cdot m^{-2} \cdot s^{-1}$. Conditions on the other dates are as given in Table 4. Standard error and number of replicates are given in parentheses. An asterisk indicates that the treatment was significantly different from the control ($P < 0.05$).

Date	Station	Control	Treatment
4 October 1984	WP1	0.244 (0.061, 4)	0.337 (0.147, 4)
	WP3	0.251 (0.149, 4)	0.331 (0.329, 4)
6 November 1984	WP1	0.145 (0.084, 5)	0.187 (0.059, 5)
	WP3	0.199 (0.070, 5)	0.104 (0.024, 5)*
16 May 1985	WP1	0.277 (0.050, 5)	0.359 (0.065, 5)
	WP3	0.347 (0.037, 5)	0.343 (0.092, 5)

TABLE 6. ATP-specific phosphatase activity ($\mu mol\ NP \cdot \mu g\ ATP^{-1}$) associated with the epilithic community and pH and SRP concentrations ($\mu g\ P \cdot L^{-1}$) at each Walker Camp Prong site on 19 September 1985 and 13 November 1985. Standard error and number of replicates are given in parentheses. Phosphatase values were significantly different among all sites with the exception of WP3 and WP4 in November ($P < 0.05$).

Date	Station	pH	SRP	ATP-specific phosphatase activity
19 September 1985	WP4	4.51	1.8	1.58 (0.29, 5)
	WP3	4.83	2.1	2.59 (0.54, 5)
	WP5	5.83	0.6	5.71 (1.62, 5)
	WP1	6.71	0.7	24.0 (6.3, 5)
13 November 1985	WP4	4.52	0.9	1.91 (0.49, 4)
	WP3	4.77	<0.5	2.47 (0.41, 5)
	WP5	5.95	<0.5	8.95 (0.63, 5)
	WP1	6.33	0.7	16.8 (3.0, 5)

concentrations were increased from about 0.01 to 0.06 $mg \cdot L^{-1}$ in an acidic lake. Although these concentrations are substantially lower than those at the highly acidic sites in Walker Camp Prong, the fractional increase over ambient levels was quite high.

Results of experiments with short-term additions of PO_4 (10 $\mu g\ P \cdot L^{-1}$) to a highly acidic site (WP3) and a less acidic site (WP1) were inconclusive (Table 5). Chlorophyll-specific productivity often increased with PO_4 addition, but the increases were not significant. On one occasion, productivity at WP3 declined significantly with PO_4 addition. Depression in chlorophyll-specific productivity immediately after PO_4 addition is a commonly observed short-term response of phosphorus-limited algae (Gerhart and Likens 1975; Healey 1979).

Phosphatase activity of the epilithic community, normalized for ATP density, was highest at the least acidic site (WP1) and was lowest at the most acidic site (Table 6). Although our assay would include phosphatase produced by autotrophic and heterotrophic components of the epilithon, the data clearly indicated that at least some portion of the community is more strongly phosphorus limited at the least acidic sites (WP1 and WP5) compared with the highly acidic sites (WP4 and WP3). This may be due to lower PO_4 concentrations resulting from co-precipitation of PO_4 and aluminum as streamwater pH increases downstream from acidified reaches, as has been suggested by Hendrey et al. (1980). Our data on average annual SRP concentrations (Table 1) and SRP concentrations on the dates of the phosphatase assays (Table 6) may indicate, to some extent, lower concentrations of available phosphorus at WP5 and WP1. However, SRP probably overestimates PO_4 concentration (Rigler 1968; Stainton 1980). Differences in phosphatase activity are likely a more sensitive assay of phosphorus limitation than either SRP concentrations in water or results of short-term PO_4 enrichment studies (Vincent 1981).

Jansson (1981) has reported extremely high phosphatase activity in an acidified lake and attributed it to competitive inhibition of enzymatic hydrolysis of phosphatase substrates by high concentrations of aluminum. Jansson hypothesized that the biota respond to high aluminum concentrations by producing greater levels of phosphatase which enable the enzymes to outcompete aluminum for the substrate. In our study, we did not observe this phenomenon. The sites with highest concentrations of aluminum (WP4 and WP3) showed relatively low phosphatase activity. Perhaps the aluminum concentrations at these sites (<0.3 $mg \cdot L^{-1}$) were too low to produce appreciable competition for the organic phosphorus substrates.

Relative rates of phosphorus turnover in streamwater resulting from uptake by the epilithic community were almost twice as great at WP5 compared with WP4 on 3 December 1985 (Fig. 6). Uptake of $^{33}PO_4$ by suspended particles was low for each site (≤2% of ^{33}P added), and a mass balance of ^{33}P after 20 h of incubation indicated that virtually all of the ^{33}P removed from the water was taken up by the epilithic community. The fraction of ^{33}P taken up into structural pools (as indicated by the EDTA extraction) compared with labile pools (H_3PO_4 extraction) (see Methods) was greater at WP5 (63% of total ^{33}P uptake into structural pools) than at WP4 (48% into structural pools). SRP concentrations on this date were greater at WP5 (2.4 $\mu g \cdot L^{-1}$) than at WP4 (1.0 $\mu g \cdot L^{-1}$) and therefore

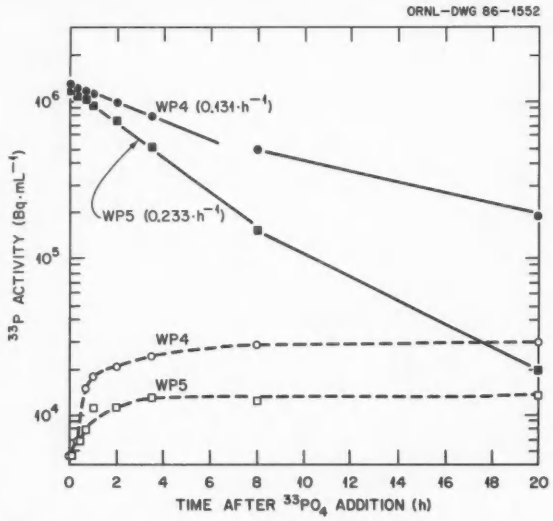


FIG. 6. Turnover of tracer additions of $^{33}PO_4$ in water from highly acidic (WP4) and slightly acidic (WP5) second-order stream sites. Incubations were conducted at 10°C with 1 L of streamwater and two tiles (85.6 cm^2) colonized by periphyton from each site. Solid lines indicate ^{33}P activity in water and broken lines indicate ^{33}P on suspended particles (>0.1 μm) over time. Turnover rates were computed using data for the initial 3.5 h of the incubation.

probably overestimated PO_4 concentration considerably. Greater PO_4 turnover at WP5 and increased incorporation of ^{33}P into structural pools at this site, compared with WP4, further indicate greater PO_4 demand relative to its supply at the less acidic of these second-order stream sites. However, chlorophyll-specific production at WP5 was always greater than that at WP4 (Fig. 5) (although differences were not significant), indicating that factors other than PO_4 availability were probably more important in limiting areal primary productivity at the less acidic second-order site (WP5).

Biological Factors Controlling Periphyton Productivity

Biological factors (i.e. trophic structure) could be responsible for differences in periphyton biomass (as indicated by cell biovolume and chlorophyll *a* density) and areal primary productivity observed among the second- and third-order sites. The density of grazing macroinvertebrates, primarily grazing species of mayfly larvae, measured during April 1985 was substantially lower at the most acidic sites (WP4, WP3, and TB) compared with sites with pH ≥ 5.7 (WP5 and CO) (Table 7). The number of different grazer taxa present at all sites was low, with the exception of the highest pH second-order site (CO), and there was a shift from dominance of the grazer community by *Epeorus pleuralis* at the higher pH, second-order sites, WP5 and CO, to *Ameletus lineatus* at the highly acidic sites (WP4, WP3, and TB). Reduced grazing intensity at the highly acidic sites could be responsible for increased cell biovolume, chlorophyll *a* density, and areal primary productivity at these sites. In addition, reduced grazing intensity could be responsible, at least in part, for the increasing importance of diatoms at the highly acidic sites if these larger periphyton cells are preferentially or more efficiently grazed than the smaller sized cells.

TABLE 7. Density (number·0.1 m^{-2}) of grazing macroinvertebrates in April 1985. Values are means of samples taken at five locations at each site with standard error given in parentheses.

Station	Median pH	Grazer abundance	Number of taxa
WP4	4.5	27.8 (6.3)	3
WP3	4.8	52.8 (16.0)	4
TB	5.0	28.6 (5.6)	4
WP5	5.7	112.4 (18.4)	4
WP1	6.4	35.0 (7.5)	3
CO	6.4	222.2 (66.5)	11

Several investigators have reported reduced densities of macroinvertebrates in streams acidified by strong mineral acids, particularly when pH's are depressed below 5.0 (Hall et al. 1980; Townsend et al. 1983; Kimmel et al. 1985). Studies have also shown that reductions in densities of grazing invertebrates in streams result in increased periphyton biomass and areal primary production, despite decreases in specific metabolic activity of individual periphyton cells (e.g. Lamberti and Resh 1983; Mulholland et al. 1983). Hendrey (1976) suggested that increased biomass and production of stream periphyton under acidic conditions was the result of reduced grazing by macroinvertebrates and microheterotrophs which were less able to adapt and maintain their activity at low pH. Our results for the second- and third-order stream sites appear to support this hypothesis. The low chlorophyll *a* density and areal primary productivity at a fourth-order site (WP1), however, are not consistent with the low density of grazers at this site (Table 7), and therefore cannot be explained by the grazing hypothesis. Because WP1 drains a much greater area than the other sites (Table 1), it should have considerably greater flow. Also, streamflow during storm events is much more turbulent at WP1 compared with the other sites (personal observation). Extremely high water velocities have been shown to reduce chlorophyll accrual by periphyton communities in streams (Horner and Welch 1981). The low chlorophyll *a* density and areal primary productivity at WP1 may be the result of greater scouring forces at this site compared with the other sites studied.

Conclusions

The periphyton communities at several sites in Walker Camp Prong and two of its tributaries in the GSMNP differed greatly according to pH. The highly acidic sites (pH ≤ 5) were generally dominated by diatoms and, to some extent, green algae, whereas sites with higher pH (pH ≥ 5.7) were dominated by blue-green algae and chrysophytes. The highly acidic sites also had greater total cell biovolume, chlorophyll *a* density, and areal primary productivity. Although differences in chlorophyll-specific productivity were not significantly different among sites, lowest values were consistently measured at the most acidic site. Low chlorophyll-specific productivity at the most acidic site could be the result of self-limitation at high levels of biomass or of inhibition related to water chemistry changes with acidification.

Experiments involving short-term additions of H_2SO_4 to the site with highest pH indicated no adverse effect of pH reduction alone. Experiments involving short-term manipulation of TIC and aluminum concentrations resulted in some statistically sig-

nificant changes in chlorophyll-specific productivity which may indicate a detrimental effect of acidification (reduced TIC and increased aluminum concentrations) on periphyton metabolism, particularly at the highly acidic third-order site. Studies of phosphatase activity and PO_4 turnover indicated that two of the least acidic sites were the most phosphorus limited, perhaps a result of co-precipitation of aluminum and PO_4 as pH increased downstream from acidified reaches. However, rates of chlorophyll-specific productivity at the less acidic sites were as high or higher than those at the highly acidic sites. Therefore, increased phosphorus limitation was probably not responsible for lower levels of chlorophyll *a* density and areal primary productivity at the less acidic sites.

Hendrey (1976) suggested that periphyton biomass and areal productivity in streams increased under highly acidic conditions because densities of grazing macroinvertebrates and microheterotrophs were reduced at low pH. Our results indicating high cell biovolume, chlorophyll *a* density, and areal primary productivity at the highly acidic second- and third-order stream sites, compared with second-order sites with higher pH, support this hypothesis. Other factors must be responsible for the low chlorophyll *a* density and areal primary productivity at the much larger fourth-order site. The area drained by this site is considerably larger than the second- and third-order sites, and increased scouring of the stream bottom resulting from greater water velocities, particularly during storms, may limit the accrual of periphyton biomass to a greater extent at this site.

Acknowledgments

We are grateful to Leigh Ann Ferren and Yvonne Vadeboncoeur for assistance in the field and laboratory determinations of chlorophyll *a* and ATP, Mary Anna Bogle and Ralph Turner for water chemistry data, and Amy Rosemond for macroinvertebrate sorting and enumeration. We also thank Steve Bartell, Jeffrey Giddings, Bruce Kimmel, Charles Goldman, and Michael Turner for helpful reviews of the manuscript. We are also grateful for the cooperation of personnel of the Great Smoky Mountains National Park. This work was supported by a grant from the Electric Power Research Institute.

References

ALMER, B., W. DICKSON, C. EKSTRÖM, E. HORNSTRÖM, AND U. MILLER. 1974. Effects of acidification of Swedish lakes. Ambio 3: 30–36.

AMERICAN PUBLIC HEALTH ASSOCIATION. 1985. Standard methods for the examination of water and waste water. 12th ed. APHA, Washington, DC.

BENNETT, H. D. 1969. Algae in relation to mine water. Costanea 34: 306–328.

BLEIWAS, A. S. H., P. M. STOKES, AND M. M. OLAVESON. 1984. Six years of plankton studies in the LaCloche region of Ontario, with special reference to acidification. Verh. Int. Ver. Theor. Angew. Limnol. 22: 332–337.

BROCK, T. D. 1973. Lower pH limit for the existence of blue-green algae: evolutionary and ecological implications. Science (Wash., DC) 179: 480–483.

BURNISON, B. K. 1980. Modified dimethyl sulfoxide (DMSO) extraction for chlorophyll analysis of phytoplankton. Can. J. Fish. Aquat. Sci. 37: 729–733.

DRISCOLL, C. T. 1980. Chemical characterization of some dilute acidified lakes and streams in the Adirondack region of New York State. Ph.D. thesis, Cornell University, Ithaca, NY.

1984. A procedure for the fractionation of aqueous aluminum in dilute waters. Int. J. Environ. Anal. Chem. 16: 267–284.

ELWOOD, J. W., AND D. J. NELSON. 1972. Periphyton production and grazing rates in a stream measured with ^{32}P material balance method. Oikos 23: 295–303.

FILBIN, G. J., AND R. A. HOUGH. 1984. Extraction of ^{14}C-labeled photosynthate from aquatic plants with dimethyl sulfoxide (DMSO). Limnol. Oceanogr. 29: 426–428.

FINDLAY, S. E. G., J. L. MEYER, AND R. T. EDWARDS. 1984. Measuring bacterial production via rate of incorporation of [^3H]thymidine into DNA. J. Microbiol. Methods 2: 57–62.

GERHART, D. Z., AND G. E. LIKENS. 1975. Enrichment experiments for determining nutrient limitation: four methods compared. Limnol. Oceanogr. 20: 649–653.

GOLDMAN, J. C., W. J. OSWALD, AND D. JENKINS. 1974. The kinetics of inorganic carbon limited growth. J. Water Pollut. Control Fed. 46: 554–574.

HALL, R. J., G. E. LIKENS, S. B. FIANCE, AND G. R. HENDREY. 1980. Experimental acidification of a stream in the Hubbard Brook Experimental Forest, New Hampshire. Ecology 61: 976–989.

HEALEY, F. P. 1979. Short-term responses of nutrient-deficient algae to nutrient addition. J. Phycol. 15: 289–299.

HENDREY, G. R. 1976. Effects of pH on the growth of periphytic algae in artificial stream channels. Internal Report IR 25/76. SNSF, Oslo, Norway.

HENDREY, G. R., AND F. R. WRIGHT. 1976. Acid precipitation in Norway: Effects on aquatic fauna. J. Great Lakes Res. 2 (Suppl. 1): 192–207.

HENDREY, G. R., N. D. YAN, AND K. J. BAUMGARTNER. 1980. Responses of freshwater plants and invertebrates to acidification, p. 457–466. *In* Restoration of lakes and inland waters. U.S. Environmental Protection Agency, EPA 440/5-81-010, Washington, DC.

HERRMAN, R., AND J. BARON. 1980. Aluminum mobilization in acid stream environments, Great Smoky Mountains National Park, U.S.A., p. 218–219. *In* D. Drabløs and A. Tollan [ed.] Ecological impact of acid precipitation. SNSF, Oslo, Norway.

HOLM-HANSEN, O., AND B. RIEMANN. 1978. Chlorophyll *a* determination: improvements in methodology. Oikos 30: 438–447.

HORNER, R. R., AND E. B. WELCH. 1981. Stream periphyton development in relation to current velocity and nutrients. Can. J. Fish. Aquat. Sci. 38: 449–457.

HORNICK, L. E., J. R. WEBSTER, AND E. F. BENFIELD. 1981. Periphyton production in an Appalachian Mountain trout stream. Am. Midl Nat. 106: 22–36.

HUCKABEE, J. W., C. P. GOODYEAR, AND R. D. JONES. 1975. Acid rock in the Great Smokies: unanticipated impact on aquatic biota of road construction in regions of sulfide mineralization. Trans. Am. Fish. Soc. 104: 677–684.

JANSSON, M. 1981. Induction of high phosphatase activity by aluminum in acid lakes. Arch. Hydrobiol. 93: 32–44.

JOHNSON, N. M., C. T. DRISCOLL, J. S. EATON, G. E. LIKENS, AND W. H. MCDOWELL. 1981. Acid rain, dissolved aluminum and chemical weathering at the Hubbard Brook Experimental Forest, New Hampshire. Geochim. Cosmochim. Acta 45: 1421–1437.

JONES, H. C., J. C. NOGGLE, R. C. YOUNG, J. M. KELLY, H. OLEM, R. J. RUANE, R. W. PASCH, G. J. HYFANTIS, AND W. J. PARKHURST. 1983. Investigations of the cause of fishkills in fish-rearing facilities in Raven Fork Watershed. Tennessee Valley Authority, Division of Air and Water Resources, TVA/ONR/W-83/9, Chattanooga, TN.

KARL, D. M., AND D. B. CRAVEN. 1980. Effects of alkaline phosphatase activity on nucleotide measurements in aquatic microbial communities. Appl. Environ. Microbiol. 40: 549–561.

KEITHAN, E. D., AND R. L. LOWE. 1985. Primary productivity and spatial structure of phytolithic growth in streams in the Great Smoky Mountains National Park, Tennessee. Hydrobiologia 123: 59–67.

KIMMEL, W. G., D. J. MURPHEY, W. E. SHARPE, AND D. R. DEWALLE. 1985. Macroinvertebrate community structure and detritus processing rates in two southwester Pennsylvania streams acidified by atmospheric deposition. Hydrobiologia 124: 97–102.

KING, P. B., NEUMAN, AND J. B. HADLEY. 1968. Geology of the Great Smoky Mountains National Park, Tennessee and North Carolina. U.S. Geol. Surv. Prof. Pap. 587: 23 p.

KWIATKOWSKI, R. E., AND J. C. ROFF. 1976. Effects of acidity on the phytoplankton and primary productivity of selected northern Ontario lakes. Can. J. Bot. 54: 2546–2561.

LAMBERTI, G. A., AND V. H. RESH. 1983. Stream periphyton and insect herbivores: an experimental study of grazing by a caddis fly population. Ecology 64: 1124–1135.

1985. Comparability of introduced tiles and natural substrates for sampling lotic bacteria, algae, and macroinvertebrates. Freshwater Biol. 15: 21–30.

LAMPKIN, A. J. III, AND M. R. SOMMERFELD. 1982. Algal distribution in a small, intermittent stream receiving acid mine-drainage. J. Phycol. 18: 196–199.

MARKER, A. F. H. 1972. The use of acetone and methanol in the estimation

of chlorophyll in the presence of phaeophytin. Freshwater Biol. 2: 361–385.

McINTIRE, C. D., AND H. K. PHINNEY. 1965. Laboratory studies of periphyton production and community metabolism in lotic environments. Ecol. Monogr. 35: 237–258.

MERRITT, R. W., AND K. W. CUMMINS. 1984. An introduction to the aquatic insects of North America. 2nd ed. Kendall/Hunt Publishing Co., Dubuque, IA.

MINSHALL, G. W. 1967. Role of allochthonous detritus in the trophic structure of a woodland springbrook community. Ecology 48: 139–149.

MULHOLLAND, P. J., J. D. NEWBOLD, J. W. ELWOOD, AND C. L. HOM. 1983. The effect of grazing intensity on phosphorus spiralling in autotrophic streams. Oecologia 58: 358–366.

MULLER, P. 1980. Effects of artificial acidification on the growth of periphyton. Can. J. Fish. Aquat. Sci. 37: 355–363.

MURPHY, J., AND J. P. RILEY. 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27: 31–36.

NALEWAJKO, C., AND B. PAUL. 1985. Effects of manipulations of aluminum concentrations and pH on phosphate uptake and photosynthesis of planktonic communities in two Precambrian Shield lakes. Can. J. Fish. Aquat. Sci. 42: 1946–1953.

PALUMBO, A. V., P. J. MULHOLLAND, AND J. W. ELWOOD. 1987. Simultaneous measurement of periphyton production (^{14}C Uptake), chlorophyll, and ATP using DMSO extraction. Limnol. Oceanogr. (In press)

RIGLER, F. H. 1968. Further observations inconsistent with the hypothesis that the molybdenum blue method measures orthophosphate in lake water. Limnol. Oceanogr. 13: 7–13.

ROGERS, J. H. JR., K. L. DICKSON, AND J. CAIRNS, JR. 1978. A chamber for in situ evaluations of periphyton productivity in lotic systems. Arch. Hydrobiol. 84: 389–398.

SAS Institute. 1982. Statistical analysis system (SAS) user's guide. 1982 ed. SAS Institute, Cary, NC.

SCHINDLER, D. W., K. H. MILLS, D. F. MALLEY, D. L. FINDLAY, J. A. SHEARER, I. J. DAVIES, M. A. TURNER, G. A. LINSEY, AND D. R. CRUIKSHANK. 1985. Long-term ecosystem stress: the effects of years of experimental acidification on a small lake. Science (Wash., DC) 228: 1395–1401.

SHANKS, R. E. 1954. Climates of the Great Smoky Mountains. Ecology 35: 354–361.

SHOAF, W. T., AND B. W. LIUM. 1976. Improved extraction of chlorophyll *a* and *b* from algae using dimethyl sulfoxide. Limnol. Oceanogr. 21: 926–928.

STAINTON, M. P. 1980. Errors in molybdenum blue methods for determining orthophosphate in freshwater. Can. J. Fish. Aquat. Sci. 37: 472–478.

STEEMANN NIELSEN, E. 1952. The use of radioactive carbon (^{14}C) for measuring organic production in the sea. J. Cons. Int. Explor. Mer 18: 117–140.

STEVENSON, R. J. 1984. Procedures for mounting algae in a syrup medium. Trans. Am. Microsc. Soc. 103: 320–321.

STEVENSON, R. J., R. SINGER, D. A. ROBERTS, AND C. W. BOYLEN. 1985. Patterns of benthic algal abundance with depth, tropic status, and acidity in poorly buffered, New Hampshire lakes. Can. J. Fish. Aquat. Sci. 42: 1501–1512.

STRICKLAND, J. D. H., AND T. R. PARSONS. 1972. A practical hardbook of seawater analysis. Bull. Fish. Res. Board Can. 167: 310 p.

TOWNSEND, C. R., A. G. HILDREW, AND J. FRANCIS. 1983. Community structure in some southern English streams: the influence of physicochemical factors. Freshwater Biol. 13: 521–544.

TUCHMAN, M. L., AND R. J. STEVENSON. 1980. Comparison of clay tile, sterilized rock, and natural substrate diatom communities in a small stream in southeastern Michigan, USA. Hydrobiologia 75: 73–79.

TURNER, M. A., D. W. SCHINDLER, AND R. W. GRAHAM. 1983. Photosynthesis–irradiance relationships of epilithic algae measured in the laboratory and in situ, p. 73–87. *In* R. G. Wetzel [ed.] Periphyton of freshwater ecosystems. Dr. W. Junk, The Hague, The Netherlands.

VINCENT, W. F. 1981. Rapid physiological assays for nutrient demand by the plankton. II. Phosphorus. J. Plankton Res. 3: 699–709.

WHITTON, B. A. 1972. Toxicity of heavy metals to freshwater algae: a review. Phytos 9: 116–125.

WINTERBOURN, M. J., A. G. HILDREW, AND A. BOX. 1985. Structure and grazing of stone surface organic layers in some acid streams of southern England. Freshwater Biol. 154: 363–374.

YAN, N. D. 1979. Phytoplankton community of an acidified, heavy metal-contaminated lake near Sudbury, Ontario: 1973–1977. Water Air Soil Pollut. 11: 43–55.

Metabolic Changes during the Reproductive Migration of Two Sympatric Coregonines, *Coregonus artedii* and *Coregonus clupeaformis*

Helga Guderley, Pierre Blier, and Lorraine Richard

Département de Biologie, Université Laval, Québec (Québec) G1K 7P4

Guderley, H., P. Blier, and L. Richard. 1986. Metabolic changes during the reproductive migration of two sympatric coregonines, *Coregonus artedii* and *Coregonus clupeaformis*. Can. J. Fish. Aquat. Sci. 43: 1859–1865.

The reproductive migration from James Bay to the Eastmain River leads to more marked changes in the metabolic organization of the swimming muscle of *Coregonus artedii* than *Coregonus clupeaformis*. The glycolytic potential of both red and white muscle decreased significantly during the migration while the aerobic potential increased significantly at the end of the migration in white muscle of *C. artedii*. This increase in aerobic potential could facilitate the upstream migration of this species. In contrast, *C. clupeaformis* generally maintained its glycolytic capacity in both red and white muscle and did not increase the aerobic potential of its white muscle at the end of the migration. In both species, hepatic enzyme activities were variable. In liver of *C. artedii* the gluconeogenic potential, as judged by the ratio of fructose 1,6-bisphosphatase to phosphofructokinase 1 activity, and the transaminases glutamate-pyruvate aminotransferase and glutamate-oxaloacetate aminotransferase, increased during the spawning migration. In *C. clupeaformis* liver, most enzymes decreased significantly during the migration.

La migration de frai s'effectuant de la baie James à la rivière Eastmain se traduit par des modifications de la structure métabolique des muscles natatoires plus marquées chez *Coregonus artedii* que chez *Coregonus clupeaformis*. Chez *C. artedii*, le potentiel glycolytique des muscles rouges et blancs décroît de façon significative au cours de la migration tandis que le potentiel aérobie des muscles blancs s'accroît de façon significative vers la fin de la migration. Cette augmentation du potentiel aérobie pourrait faciliter la migration d'amont chez cette espèce. Au contraire, la capacité glycolytique des muscles rouges et blancs de *C. clupeaformis* se maintient généralement au même niveau et le potentiel aérobie des muscles blancs ne s'accroît pas vers la fin de la migration. Les activités enzymatiques hépatiques étaient variables chez les deux espèces. Chez *C. artedii*, le potentiel gluconéogène du foie, déterminé par le rapport des activités de la fructose biphosphatase-1,6 et de la phosphofructokinase 1 de même que par celles des transaminases, de la glutamate-pyruvate aminotransférase et de la glutamate-oxaloacétate aminotransférase, augmentait au cours de la migration de frai. On notait, chez *C. clupeaformis*, une baisse significative de l'activité de la plupart des enzymes hépatiques au cours de la migration.

Received January 21, 1986
Accepted June 11, 986
(J8654)

Reçu le 21 janvier 1986
Accepté le 11 juin 1986

Most species of anadromous fish rely upon their endogenous stores to fuel their reproductive migrations, to complete gonad maturation, and to adapt to the ensuing changes in salinity and temperature. If the fish survive the reproductive season, they then spend a lengthy overwintering period without food. Ambient temperature is the major factor determining the duration of overwintering and of food deprivation. Thus, for numerous northern fish species, the anorexic period is considerably longer than the feeding period. In contrast with mammals in which food deprivation brings about rapid glycogen depletion (Hers and Hue 1983), at least some fish species respond to starvation with preferential use of lipid or protein stores and conservation of their hepatic glycogen stores even during prolonged fasting (Love 1980). The metabolic response to food deprivation during a spawning migration has been examined only for migrating sockeye salmon (*Oncorhynchus nerka*) in which the enzymes required for alanine synthesis are selectively maintained in white muscle despite a generalized decrease in glycolytic enzymes, Krebs cycle enzymes, and protein levels (Mommsen et al. 1980). Isolated hepatocytes from these salmon maintain high rates of gluconeogenesis from alanine throughout the migration (French et al. 1983). This presumably permits the conservation of hepatic glycogen levels throughout their spawning migration. However, the metabolic changes which accompany reproductive migrations for species which survive these migrations are not known.

In the current study, we examine the metabolic organization of liver and swimming musculature in anadromous coregonine populations during their spawning migration between James Bay and the Eastmain River in northern Quebec (Fig. 1). *Coregonus artedii* and *C. clupeaformis* leave their feeding grounds in James Bay in late July, spend 2 and 6 wk, respectively, in the estuarine portion of the river, and complete their upstream migration before the end of September. Spawning occurs 6–10 wk after completion of this migration. While

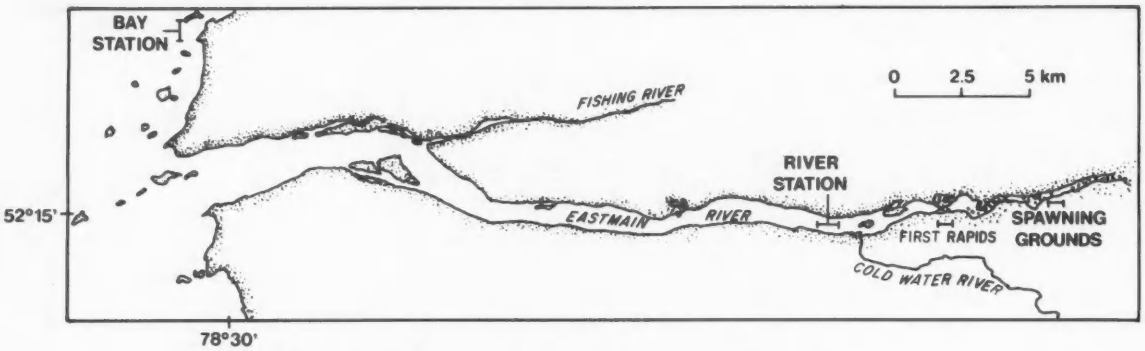


FIG. 1. Eastmain River showing the location of the sampling stations.

both species survive spawning, considerable mortality occurs between spawning and the spring seaward migration, particularly for *C. artedii* (Dodson et al. 1986). For both species, the energetic cost/distance migrated is high, largely due to the lengthy time spent migrating and waiting at the spawning grounds (L. Bernatchez and J. J. Dodson, Département de biologie, Université Laval, pers. comm.). As *C. artedii* has a lower aerobic scope for activity than *C. clupeaformis* (Bernatchez and Dodson 1985), the range of speeds supported by aerobic metabolism is more limited for *C. artedii* than for *C. clupeaformis*. Bernatchez and Dodson (1985) indicated that the detrimental effect of low temperature on swimming performance leads both species to migrate upstream before temperatures decrease. As *C. artedii* is smaller, the theoretical minimal temperature for crossing the rapids is higher than for *C. clupeaformis* (Dodson et al. 1986). While *C. artedii* is abundant at this location, it is closer to its northern limit of distribution than *C. clupeaformis* (Morin et al. 1980). The feeding strategy of the two species also differs: *C. artedii* have empty stomachs in the river, while approximately half of the *C. clupeaformis* had full stomachs during the initial half of the migration (Dodson et al. 1986). Since *C. artedii* does not feed during its migration and seems more limited by low temperatures than *C. clupeaformis*, we hypothesize that *C. artedii* changes its metabolic organization more markedly than *C. clupeaformis* during the migration.

Given the remoteness of the Eastmain River, we worked with frozen tissues and monitored the metabolic organization of liver and swimming musculature by measuring maximal enzyme activities. Measurements of enzyme activities are commonly used to elucidate the metabolic orientation of tissues (Newsholme et al. 1978; Moon and Johnston 1980; Mommsen et al. 1980; Moon 1983; Blier and Guderley 1986). In red and white muscle, we measured certain enzymes of glycolysis, of the Krebs cycle, and of amino acid metabolism. In liver, we also measured certain gluconeogenic enzymes. For the enzymes under study, the maximal activities are relatively independent of changes in the levels of allosteric effectors and phosphorylation effects (Hers and Hue 1983). While measurements of maximal enzyme activities reveal quantitative changes in metabolic pathways during long-term adaptational processes, they cannot reveal changes in metabolic flux. Similarly, measurements in frozen tissues permit comparisons among similarly treated samples, but do not reliably indicate enzyme levels in vivo.

Materials and Methods

Experimental Animals

Five to eight adult *Coregonus artedii* and *C. clupeaformis* were captured at three stations: (1) near the east coast of James Bay (Bay Station), (2) 25 km upstream in the estuarine portion of the Eastmain River (River Station), and (3) at the spawning grounds, 33 km from the river's mouth (Spawning Grounds) (Fig. 1). Samples were taken at the Bay Station in mid-July at a water temperature of 12–13°C, at the River Station in mid-August at a water temperature of 17–19°C, and at the Spawning Grounds in early September at a water temperature of 15–17°C. At the latter, the fish were sampled shortly after their arrival and well before spawning. At the Bay and River stations, the fish were placed on ice after capture with gill nets and frozen at −20°C within 1 h of removal from water. However, due to the remoteness of the Spawning Grounds, these samples were transported for 2 h before freezing. While this increased time before freezing could have led to an underestimation of enzyme levels, inspection of the data shows that this did not mask numerous increases in enzyme levels (see Results). The frozen samples were flown to our laboratory at Université Laval, for analysis. The fish were kept at −20°C until analysis. Both male and females were used in this study, and while we found no variation in enzyme activities with sex, the small sample size prevented a rigorous comparison. We did not measure the hepatosomatic or gonadal index of the fish, as this would have required complete thawing of the specimens and would have been detrimental to our schedule of enzyme determinations. Such data are being obtained by Y. Lambert and J. J. Dodson (Département de biologie, Université Laval, pers. comm.) in their study of the energy budget of these species during their migration in this river.

Preparation of Tissue Extracts

The fish were partially thawed and small samples of white muscle, red muscle, and liver were removed and placed in 3 mL/g of the homogenization buffer (50 m*M* Imidazole–HCl, 50 m*M* KCl, 150 m*M* sucrose, 1 m*M* EDTA, 2 m*M* glutathione, pH 6.8). White muscle was sampled well under the dorsal fin to avoid contamination by red muscle. Red muscle was sampled along the lateral line of the fish. The samples were homogenized for three 30-s periods with a Polytron, with cooling between each period. The homogenates

were centrifuged at 15 000 × *g* for 30 min in a refrigerated centrifuge (0–4°C) and enzyme activities were measured in the supernatants. Total protein concentrations were determined in additional extracts of white muscle and liver using the method of Lowry et al. (1951).

Measurements of Enzyme Activity

Enzyme activities were determined in a Varian Cary UV/Vis spectrophotometer in which the sample chamber was maintained at 15°C by a Haake refrigerated circulating water bath. Enzyme activities were measured by following the absorbance of NADH or NADPH at 340 nm, except for citrate synthase which was measured at 412 nm to monitor the transfer of sulfhydryl groups to 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB). The pH of the assay mixtures was adjusted at 25°C. The optimality of assay conditions was determined for white muscle enzymes from *C. artedii* and the same conditions were used for both species. White muscle enzymes were measured as follows.

Phosphofructokinase (EC 2.7.1.11) (PFK): 50 m*M* Tris–HCl, 100 m*M* KCl, 10 m*M* $MgCl_2$, 0.16 m*M* NADH, 10 m*M* ATP, 4 m*M* fructose 6-phosphate (omitted for control), pH 8.0. Excess levels of aldolase, triose-phosphate isomerase, and α-glycerol phosphate dehydrogenase were added.

Fructose 1,6-bisphosphatase (EC 3.1.3.11) (FBPase): 50 m*M* Imidazole–HCl, 6 m*M* $MgCl_2$, 0.4 m*M* NADP, 0.1 m*M* fructose 1,6-bisphosphate (omitted for control), pH 7.0. Excess levels of phosphoglucose isomerase and glucose 6-phosphate dehydrogenase were added to the reaction mixture.

Pyruvate kinase (EC 2.7.1.40) (PK): 50 m*M* Imidazole–HCl, 10 m*M* $MgCl_2$, 100 m*M* KCl, 0.16 m*M* NADH, 1.3 m*M* phosphoenolpyruvate (omitted for control), 2.0 m*M* ADP, pH 7.0. Excess levels of lactate dehydrogenase were added.

Lactate dehydrogenase (EC 1.1.1.27) (LDH): 100 m*M* potassium phosphate, 0.4 m*M* NADH, 0.6 m*M* pyruvate (omitted for control), pH 7.0.

Citrate synthase (EC 4.1.3.7) (CS): 100 m*M* Tris–HCl, 0.1 m*M* DTNB, 0.3 m*M* acetyl CoA, 0.5 m*M* oxaloacetate (omitted for control), pH 8.0.

Malate dehydrogenase (EC 1.1.1.37) (MDH): 50 m*M* Imidazole–HCl, 0.2 m*M* NADH, 0.65 m*M* oxaloacetate (omitted for control), pH 7.0.

Malic enzyme (EC 1.1.1.40) (ME): 50 m*M* Imidazole–HCl, 4 m*M* $MgCl_2$, 0.4 m*M* NADP, 1 m*M* malate (omitted for control).

Glutamate-oxaloacetate aminotransferase (EC 2.6.1.1) (GOT): 100 m*M* potassium phosphate, 0.2 m*M* NADH, 15 m*M* α-ketoglutarate, 30 m*M* aspartate (omitted for control), pH 7.0. Excess levels of MDH were added.

Glutamate-pyruvate aminotransferase (EC 2.6.1.2) (GPT): 100 m*M* potassium phosphate, 0.2 m*M* NADH, 10 m*M* α-ketoglutarate, 2.6 m*M* alanine (omitted for control), pH 7.0. Excess levels of LDH were added.

PK, LDH, MDH, GOT, GPT, and CS were measured in extracts of red muscle using the above conditions. The enzymes measured in white muscle were also measured in liver with the addition of glutamate dehydrogenase and phosphoenolpyruvate carboxykinase for which the optimal conditions were as follows.

Glutamate dehydrogenase (EC 1.4.1.3) (GDH): 50 m*M* Imidazole–HCl, 250 m*M* ammonium acetate, 0.1 m*M* EDTA, 0.1 m*M* NADH, 1 m*M* ADP, 14 m*M* α-ketoglutarate (omitted for control), pH 7.0.

Phosphoenolpyruvate carboxykinase (EC 4.1.1.32) (PEPCK): 50 m*M* Tris–HCl, 1.0 m*M* $MnCl_2$, 0.1 m*M* NADH, 0.3 m*M* deoxy-GDP, 0.5 m*M* phosphoenolpyruvate, 60 m*M* $NaHCO_3$, (omitted for control and replaced by 60 m*M* NaCl), pH 7.0. Excess levels of MDH were added.

For PFK, the optimal conditions for the measurement in liver extracts differed from those in muscle extracts: 50 m*M* Tris–HCl, 100 m*M* KCl, 10 m*M* $MgCl_2$, 0.16 m*M* NADH, 2.5 m*M* ATP, 5 m*M* fructose 6-phosphate (omitted for control), 2.0 m*M* phosphoenolpyruvate, pH 8.0. Excess pyruvate kinase and lactate dehydrogenase were added.

Calculation and Statistical Analysis

All reactions were linear over the period used for calculation of enzyme activity. The reactions were started by addition of tissue extract to the reaction mixture. One unit of activity is the amount of enzyme producing 1 μmol of product per minute at 15°C. Enzyme activities are expressed as mean units per gram frozen weight. We also expressed enzyme activities as units per milligram of protein for liver and white muscle. Statistical comparisons were made using the Mann–Whitney *U* test. Statistical comparisons of the liver and white muscle enzyme activities at the different stations showed similar results whether the data were expressed as units per gram of frozen tissue or units per milligram of protein. We found that expressing the data as units per gram of frozen tissue gave somewhat more conservative estimates of differences among stations. Since freezer failure prevented us from measuring the protein concentration of red muscle, all data are expressed as units per gram of frozen tissue. The few cases where expressing the data as units per gram of frozen tissue indicated greater differences among stations are noted in the text. Particularly for certain freezing-sensitive enzymes, such as PFK, FBPase, and PEPCK (Cryer and Bartley 1974), the values should not be used to evaluate in vivo levels of the enzymes but rather changes in these enzymes among stations.

Chemicals

Biochemicals and coupling enzymes were purchased from Sigma Chemicals (St. Louis, MO). Other chemicals were reagent grade.

Results

Coregonus artedii from the three stations along their migration did not differ ($p > 0.05$) in their forklengths (Bay 27.7 ± 0.8 cm (5), River 27.9 ± 1.0 cm (5), Spawning Grounds 27.0 ± 1.5 cm (4)). *Coregonus clupeaformis* also showed no interstation differences ($p > 0.05$) in their forklengths (Bay 31.1 ± 1.5 cm (5), River 32.5 ± 1.3 cm (5), Spawning Grounds 33.0 ± 2.6 cm (5)). Thus, interstation intraspecies comparisons of enzyme activities can be made without concern for the confounding effects of scaling (Sullivan and Somero 1983).

Changes in Muscle during Migration

Two distinct patterns of variation of enzyme activity in the white muscle of *C. artedii* during its upstream migration become apparent upon examination of Fig. 2a–2c. The

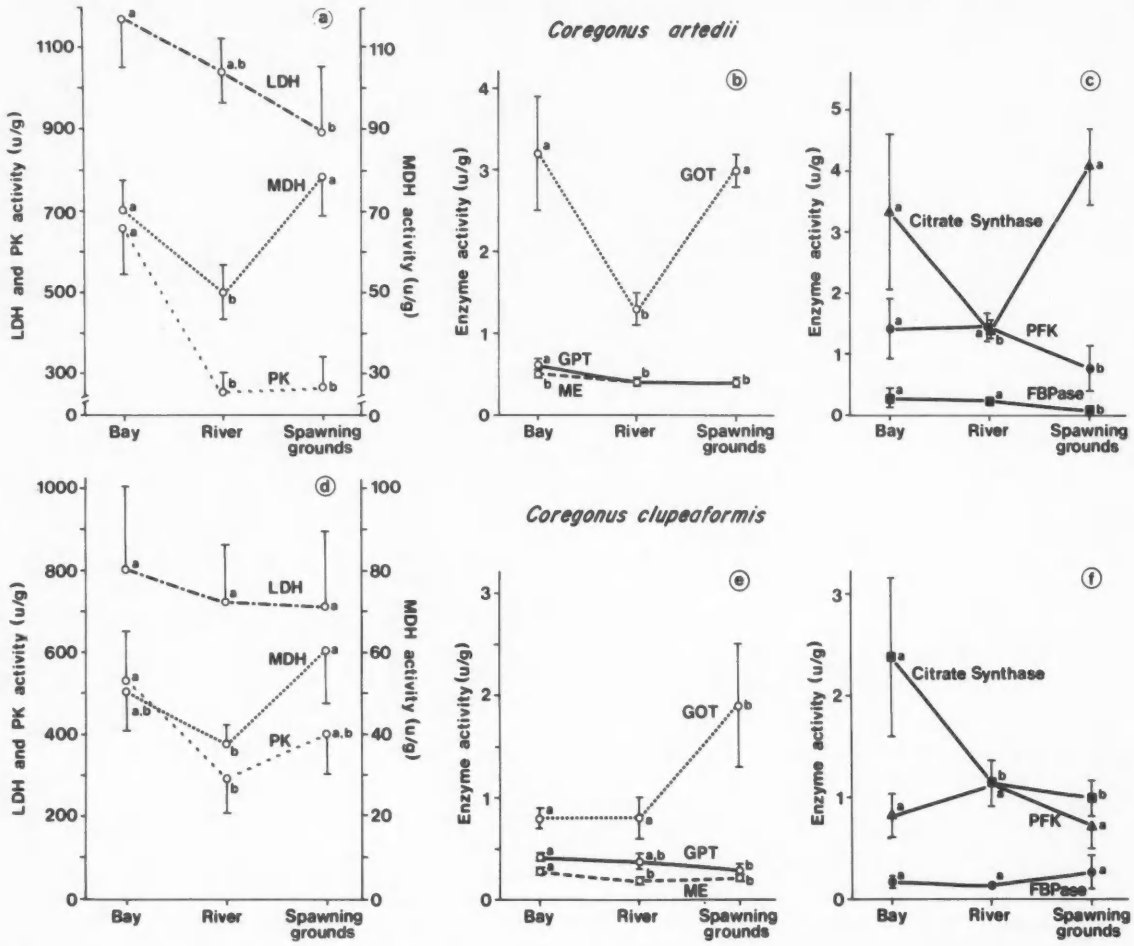


FIG. 2. Changes in enzyme activities in white muscle of (a–c) *C. artedii* and (d–f) *C. clupeaformis* during their reproductive migration in the Eastmain River. Values are shown as $X \pm$ SD. If two values of a given enzyme are followed by different letters, they are significantly different at the significance level indicated in the text. Comparisons were carried out using the Mann–Whitney U test. If two values of a given enzyme are followed by the same letter, they are not significantly different ($p > 0.05$).

glycolytic enzymes and those closely related to glycolysis, LDH, PK, PFK, FBPase, and GPT, decreased significantly ($p < 0.05$ for LDH and PFK, $p < 0.01$ for PK, GPT, and FBPase) during the migration. By contrast, several enzymes of, or related to, the Krebs cycle (CS, MDH, and GOT) first decreased and then increased during the migration ($p < 0.01$). ME (Fig. 2c) and the ratio of PFK to FBPase (Bay 5.78 ± 2.85 (6), River 6.86 ± 3.01 (5), Spawning Grounds 10.36 ± 6.22 (4)) did not change ($p > 0.05$) during the migration. Despite the marked decrease of glycolytic enzymes, protein concentration did not change ($p > 0.05$) during the migration (Bay 180.6 ± 15.2 mg/g frozen weight (5), River 194 ± 12.3 mg/g frozen weight (5), Spawning Grounds 190.3 ± 20.8 mg/g frozen weight (5)).

In red muscle of *C. artedii*, the glycolytic enzymes, PK, LDH, and GPT, also decreased during the migration (Table 1). While the Krebs cycle enzymes showed similar trends as in white muscle, the intrastation variability was greater than that found for white muscle and masked differences among stations. This variability may reflect the difficulty of isolating pure samples of red muscle.

The metabolic organization of the swimming musculature changed less dramatically during the upstream migration of *C. clupeaformis* than of *C. artedii* (Fig. 2d–2f). In white muscle, no decrease in glycolytic potential was associated with the migration, as LDH, PFK, and FBPase did not change, while PK decreased and then returned to its initial levels ($p < 0.01$). GPT decreased during the migration ($p < 0.01$), but when it was expressed in terms of units per milligram of protein, it remained constant. Despite the lack of change in PFK and FBPase, the ratio of PFK to FBPase activity increased significantly ($p < 0.01$) between the bay and river, while the value at the spawning grounds did not differ from those at the bay or river (Bay 5.06 ± 1.08 (7), River 8.66 ± 4.32 (7), Spawning Grounds 4.9 ± 4.3 (5)). Among the enzymes of, or related to, the Krebs cycle, MDH and GOT were elevated in

TABLE 1. Enzyme activities in red muscle. Data are shown as $X \pm$ SD (n) where n = the number of fish measured. Different letters after the values indicate significant differences among the activities at the different stations. The Mann–Whitney U test was used to compare the values, and the probability that the values differ is given at the end of each line. If two values are followed by the same letter, they do not differ ($p > 0.05$).

	Bay	River	Spawning Grounds	
C. artedii				
LDH	903.6±68.8 (5) a	791.3±100.3 (5) ab	706.2±58.0 (5) b	$p < 0.01$
PK	77.6±10.3 (5) a	55.7±12.6 (5) b	57.1±13.9 (5) b	$p < 0.05$
GPT	0.8±0.2 (5) ab	0.6±0.1 (5) a	0.9±0.2 (5) b	$p < 0.05$
GOT	1.5±0.4 (5) a	1.4±0.3 (5) a	1.5±0.2 (5) a	
MDH	104.1±30.4 (5) a	73.2±23.1 (5) a	88.6±13.7 (5) a	
CS	7.5±4.5 (5) a	3.6±2.1 (5) a	5.1±1.6 (5) a	
C. clupeaformis				
LDH	577.3±148.9 (5) a	454.8±53.6 (5) a	443.0±138.3 (5) a	
PK	82.4±21.6 (5) a	62.9±11.5 (5) ab	51.1±25.4 (5) b	$p < 0.05$
GPT	0.4±0.1 (5) a	0.6±0.2 (5) b	0.5±0.2 (5) ab	$p < 0.05$
GOT	0.8±0.3 (5) a	0.9±0.6 (5) ab	1.4±0.4 (5) b	$p < 0.05$
MDH	163.2±64.5 (5) a	198.7±46.3 (5) a	144.5±51.1 (5) a	
CS	4.7±3.0 (5) a	8.9±2.0 (5) b	4.0±2.1 (5) a	$p < 0.05$

TABLE 2. Enzyme activities in liver. Data are shown and compared as explained in Table 1.

	Bay	River	Spawning Grounds	
C. artedii				
LDH	224.7±128.7 (4) a	372.5±111.2 (4) b	217.7±146.1 (4) a	$p < 0.05$
PK	24.8±7.2 (4) a	23.7±16.4 (4) a	22.2±15.6 (4) a	
PFK	1.6±0.3 (4) a	1.4±0.8 (5) ab	1.1±0.3 (4) b	$p < 0.05$
GPT	1.9±1.5 (4) ab	0.9±0.3 (4) a	2.2±1.0 (4) b	$p < 0.05$
GOT	1.7±0.7 (4) ab	1.1±0.3 (4) a	2.9±1.5 (4) b	$p < 0.05$
MDH	444.6±254.4 (4) a	451.8±209.0 (4) a	582.7±286.2 (4) a	
GDH	77.4±56.3 (4) a	80.8±46.6 (4) a	93.2±63.3 (4) a	
ME	2.5±0.6 (4) a	3.3±1.0 (4) a	2.0±0.03 (4) a	
FBPase	1.4±0.3 (4) a	2.1±1.2 (4) a	1.7±0.3 (4) a	
PEPCK	0.7±0.2 (3) a	1.5±1.4 (5) a	0.5±0.3 (4) a	
FBPase/PFK	0.83±0.28 (4) a	1.28±0.23 (4) b	1.65±0.60 (4) b	$p < 0.05$
C. clupeaformis				
LDH	132.1±33.2 (5) a	171.0±47.4 (5) a	88.9±32.0 (4) b	$p < 0.01$
PK	38.2±23.3 (5) ab	31.0±6.0 (5) a	15.6±6.3 (4) b	$p < 0.01$
PFK	1.9±0.3 (5) a	2.1±0.6 (5) a	1.8±0.6 (4) a	
GPT	2.0±0.9 (5) a	1.1±0.2 (5) b	1.8±0.9 (4) ab	$p < 0.05$
GOT	3.0±1.0 (5) a	1.8±0.2 (5) b	1.7±0.6 (4) b	$p < 0.05$
MDH	537.0±96.2 (5) a	295.0±55.7 (5) b	262.5±74.4 (4) b	$p < 0.01$
GDH	89.2±15.3 (5) a	49.0±11.3 (5) b	30.6±13.8 (4) c	$p < 0.01$
ME	4.8±2.3 (5) a	1.7±0.7 (5) b	1.2±0.7 (4) b	$p < 0.01$
FBPase	1.9±0.4 (5) a	1.8±1.0 (5) ab	1.6±0.3 (4) b	$p < 0.05$
PEPCK	0.7±0.2 (5) a	0.9±0.3 (5) a	0.7±0.2 (4) a	
FBPase/PFK	1.03±0.23 (5) a	1.0±0.24 (5) a	1.01±0.66 (4) a	

fish sampled at the spawning grounds ($p < 0.01$), while CS and ME decreased during the migration ($p < 0.01$). The protein concentration of white muscle was significantly lower ($p < 0.01$) at the spawning grounds than at the other two stations (Bay 198.2 ± 21.3 mg/g frozen weight (5), River 189.2 ± 12.5 mg/g frozen weight (5), Spawning Grounds 133.6 ± 19.3 mg/g frozen weight (5)).

In red muscle of *C. clupeaformis*, LDH and MDH remained constant throughout the migration, while PK levels declined (Table 1). CS increased at the river station and decreased at the spawning grounds. The two transaminases, GPT and GOT, increased during the migration.

Metabolic Changes in Liver

Hepatic enzyme activities were highly variable in *C. artedii* (Table 2) with a mean coefficient of variation of 46.4 ± 20.5% (3) for the 10 enzymes measured at the three stations. Despite this variability, GPT and GOT levels were higher at the spawning grounds than at the river station. However, GPT levels expressed in terms of units per milligram of protein remained constant throughout the migration. The decrease in PFK levels during the migration increased the ratio of FBPase to PFK activity, an indicator of gluconeogenic potential. LDH levels increased at the river station and then returned to initial levels at the spawning grounds. Hepatic protein concentration

did not change during the migration ($p > 0.05$) (Bay 91.3 ± 13.6 mg/g frozen weight (5), River 97.2 ± 14.6 mg/g frozen weight (5), Spawning Ground 102.7 ± 18.8 mg/g frozen weight (5)). At the bay station all specimens were males, at the river station two were females, while at the spawning grounds one was a female.

Hepatic enzyme activities were less variable in *C. clupeaformis* (Table 2) than in *C. artedii*, the mean coefficient of variation being 32.3 ± 13.3% (30). Most enzymes (LDH, PK, GOT, MDH, GDH, ME, and FBPase) decreased during the migration. GPT was low in the samples from the river station and increased at the spawning grounds. However, when FBPase and GPT levels were expressed as units per milligram of protein, there were no differences among stations. Finally, PFK, PEPCK, and the ratio between FBPase and PFK activity remained constant during the migration. Hepatic protein concentrations were significantly higher at the spawning grounds than at the river station ($p < 0.01$) (Bay 123.3 ± 33.0 mg/g frozen weight (5), River 104.9 ± 14.6 mg/g frozen weight (5), Spawning Grounds 133.9 ± 6.7 mg/g frozen weight (5)). At the bay station two specimens were females, at the river station all specimens were males, while at the spawning grounds one was a female.

Discussion

Although anadromous *Coregonus artedii* and *C. clupeaformis* migrate simultaneously from James Bay to their spawning grounds on the Eastmain River, their metabolic adjustments to this migration differ markedly. As predicted, the metabolic adjustments of *C. artedii* were more dramatic, particularly in white muscle. Despite a constant protein concentration, the glycolytic potential declined markedly during the migration, while the activities of Krebs cycle enzymes, an indicator of aerobic potential, first declined and then increased. In red muscle, glycolytic enzyme activities also declined, and although the changes of Krebs cycle enzymes were not significant, they resembled those in white muscle. Simultaneously, the hepatic potential for gluconeogenesis, as estimated by the ratio of FBPase to PFK, increased as did hepatic levels of the transaminases GPT and GOT. These changes suggest selective muscle proteolysis with hepatic use of amino acids for gluconeogenesis. However, since the liver decreases in size during the migration (Dodson et al. 1986), the hepatic capacity for gluconeogenesis may remain constant. For these reproductively maturing fish, liberation of muscle amino acids could also support protein synthesis for gonad maturation. Effectively, a decreased weight of muscle, liver, and digestive tract is mirrored by an increased gonad weight during the prespawning period, particularly in females (Dodson et al. 1986).

Since *C. artedii* does not feed during its migration, food deprivation could cause these changes in enzyme activity. In white muscle of rainbow trout (*Salmo gairdneri*), starvation decreases protein synthesis and initially increases the rate of protein degradation (Loughna and Goldspink 1984). While such changes would explain the decrease in glycolytic enzymes, they would not explain the increase of aerobic enzymes in white muscle of *C. artedii* at the end of the migration. During the lengthy spawning migration of sockeye salmon in the Fraser River, the glycolytic (LDH, PK, hexokinase, and FBPase) and aerobic enzymes (MDH, CS, GOT, and GDH) in white muscle decrease simultaneously (Mommsen et al. 1980). However, since red muscle changes less than white muscle with long-term starvation (Patterson et al. 1974; Mommsen et al. 1980; Loughna and Goldspink 1984), the increase in aerobic potential at the spawning grounds could represent a sparing of aerobic fibers adjacent to the white muscle. While we cannot rule this out, we tried to avoid contamination of white muscle with intermediate or red fibers by removing only deep white muscle. Furthermore, as red and white muscle showed similar changes in their glycolytic and aerobic enzymes during the migration, we feel that food deprivation cannot explain the increased aerobic potential observed at the end of the migration.

We suggest that the increased aerobic capacity of the swimming musculature of *C. artedii* at the spawning grounds is an adaptation to facilitate their upstream migration during which they must attain speeds of roughly 1.52 m/s in the fastest rapids. This speed is equivalent to 5.2 body lengths/s for the average adult *C. artedii*. The difficulty of this migration for *C. artedii* is emphasized by the calculation of Dodson et al. (1986), which suggests that this species cannot surmount these rapids below 7°C. If *C. artedii* can provide adequate supplies of oxygen to the swimming musculature (Rome et al. 1985), the increase in the aerobic potential of muscle should decrease the anaerobic activity required to traverse the rapids. The importance of reducing anaerobic activity is emphasized by these species' avoidance of high current speeds and of low temperatures (Bernatchez and Dodson 1985). Because activity that leads to exhaustion markedly increases potential mortality (Priede 1977; Bernatchez and Dodson 1985), increases in aerobic potential and decreased use of anaerobic activity would be of selective advantage. Examination of fish captured just before they mount the rapids (i.e. at the river station in early September) would confirm whether this increased aerobic potential is present before the migration across the rapids.

The increase in the aerobic potential of white, and probably red, muscle in *C. artedii* at times when environmental temperature is dropping could be seen as anticipatory thermal compensation. An increased aerobic capacity of swimming musculature in response to low temperatures has been shown for several fish species in laboratory acclimation experiments (Johnston and Lucking 1978; Sidell 1980; Johnston and Maitland 1980; Johnston 1982; Rome et al. 1984, 1985; Tyler and Sidell 1984) and in naturally acclimatized pike (*Esox niger*) (Kleckner and Sidell 1985). Again, an increased aerobic potential would be of the greatest selective advantage for *C. artedii* during its upstream migration, as it would provide a greater thermal latitude and extend the potential migratory period. However, the signal inducing this increase in aerobic potential would not be low temperature, but may be decreasing temperature or photoperiod.

In white muscle of *C. clupeaformis*, glycolytic enzyme levels changed little with the migration, while the enzyme that is the most diagnostic for aerobic capacity, CS, was lower at the river station and the spawning grounds than at the bay station. An increased glycolytic orientation, as indicated by the ratio of PFK to FBPase, characterized the fish at the river station, but was not maintained throughout the migration. In red muscle, LDH and MDH stayed constant, while PK and CS showed inverse patterns of change during the migration. The general constance of metabolic organization at the first two

stations corresponds with the feeding of this species in the estuarine portion of the river. At the spawning grounds, where the animals are no longer feeding, white muscle protein concentration decreased. The increases of GOT and MDH in white muscle and of GOT and GPT in red muscle could facilitate the metabolism of amino acids liberated from muscle protein. The decrease in protein concentration that occurred in white muscle of *C. clupeaformis* and not in *C. artedii* may reflect the somewhat higher somatic investment made by *C. clupeaformis* in their migration (Dodson et al. 1986). Increases in the aerobic potential of the swimming musculature seem less important for *C. clupeaformis* than for *C. artedii*. Because the aerobic scope of activity and the swimming efficiency are considerably greater for *C. clupeaformis* than for *C. artedii* (Bernatchez and Dodson 1985), the former species may not require the compensatory adjustments of metabolic organization needed by the latter. Furthermore, the greater size of *C. clupeaformis* may permit this species to maintain its migratory activities at lower temperatures in the Eastmain River and permit it to extend further north than *C. artedii* (Dodson et al. 1986).

In light of the relative constance of metabolic organization in the swimming musculature of *C. clupeaformis*, the generalized decrease of hepatic enzyme activities seems anomalous. This decrease occurs despite an increase in hepatic protein concentration between the river and the spawning grounds. The decreases in enzyme levels may reflect a resorption of liver during the spawning migration. Effectively, the hepatosomatic index drops between September and October, concomitant with gonadal development (Y. Lambert and J. J. Dodson, pers. comm.).

In conclusion, as we hypothesized, the metabolic changes in *C. artedii* were more pronounced than those in *C. clupeaformis*. As *C. artedii* is smaller, closer to the northern limits of its distribution, and does not feed during its reproductive migration, it apparently must resort to more drastic metabolic reorganization than *C. clupeaformis*. We interpret the increased aerobic capacity of the swimming muscle in *C. artedii* as an adaptation facilitating its upstream migration and extending the period in which this migration is feasible. Such metabolic changes may also be used by *C. clupeaformis* in regions closer to its northern limit of distribution. Effectively, as an environment becomes increasingly rigorous, a species may need to resort to more extensive metabolic reorganization to fulfill the requirements of its life cycle. However, the metabolic changes in *C. artedii* and *C. clupeaformis* during their spawning migration are less drastic than those occurring during the Fraser River spawning migration of sockeye salmon (Mommsen et al. 1980) and suggest that species that survive their reproductive migrations use qualitatively different metabolic strategies to fuel these migrations.

Acknowledgements

Particular thanks are due to L. Bernatchez who made this study possible by collecting and freezing the specimens. J. Dodson, L. Bernatchez, J. H. Himmelman, and Y. Lambert gave critical comments on an earlier version of the manuscript. The authors express their sincere gratitude to C. Dallaire for the expert preparation of the figures. P.B. was supported by a summer scholarship from NSERC while carrying out part of this study. This study was supported by a grant from NSERC to H.G.

References

BERNATCHEZ, L., AND J. J. DODSON. 1985. Influence of temperature and current speed on the swimming capacity of lake whitefish (*Coregonus clupeaformis*) and cisco (*C. artedii*). Can. J. Fish. Aquat. Sci. 42: 1522–1529.

BLIER, P., AND H. GUDERLEY. 1986. The enzymatic and metabolic effects of extended food deprivation in *Rana pipiens*. Physiol. Zool. 59: 230–239.

CRYER, A., AND W. BARTLEY. 1974. The effect of storage on enzyme activities in tissues. Biochem. J. 144: 433–434.

DODSON, J. J., Y. LAMBERT, AND L. BERNATCHEZ. 1986. Comparative migratory and reproductive strategies of the sympatric anadromous coregonine species of James Bay. *In* M. A. Rankin [ed.] Migration: mechanisms and adaptive significance. Contrib. Mar. Sci. Suppl. 27. (In press)

FRENCH, C. J., P. W. HOCHACHKA, AND T. P. MOMMSEN. 1983. Metabolic organization of liver during spawning migration of sockeye salmon. Am. J. Physiol. 245: R827–R830.

HERS, H. G., AND L. HUE. 1983. Gluconeogenesis and related aspects of glycolysis. Annu. Rev. Biochem. 52: 617–653.

JOHNSTON, I. A. 1982. Capillarisation, oxygen diffusion distances and mitochondrial content of carp muscles following acclimation to summer and winter temperatures. Cell Tissue Res. 222: 325–337.

JOHNSTON, I. A., AND M. LUCKING. 1978. Temperature induced variation in the distribution of different types of muscle fiber in the goldfish (*Carassius carassius*, L.). J. Comp. Physiol. 124: 111–116.

JOHNSTON, I. A., AND B. MAITLAND. 1980. Temperature acclimation in crucian carp, *Carassius carassius* L., morphometric analyses of muscle fiber ultrastructure. J. Fish Biol. 17: 113–125.

KLECKNER, N. W., AND B. D. SIDELL. 1985. Comparison of maximal activities of enzymes from tissues of thermally-acclimated and naturally acclimatized chain pickerel (*Esox niger*). Physiol. Zool. 58: 18–30.

LOUGHNA, P. T., AND G. GOLDSPINK. 1984. The effects of starvation upon protein turnover in red and white myotomal muscle of rainbow trout, *Salmo gairdneri* Richardson. J. Fish Biol. 25: 223–230.

LOVE, R. M. 1980. The chemical biology of fishes II. Academic Press, London.

LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, AND R. J. RANDALL. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275.

MOMMSEN, T. P., C. J. FRENCH, AND P. W. HOCHACHKA. 1980. Sites and patterns of protein and amino acid utilization during the spawning migration of salmon. Can. J. Zool. 58: 1785–1799.

MOON, T. W. 1983. Metabolic reserves and enzyme activities with food deprivation in immature American eels, *Anguilla rostrata* (LeSueur). Can. J. Zool. 61: 802–811.

MOON, T. W., AND I. A. JOHNSTON. 1980. Starvation and the activities of glycolytic and gluconeogenic enzymes in skeletal muscles and liver of the plaice, *Pleuronectes platessa*. J. Comp. Physiol. 136: 31–38.

MORIN, R., J. J. DODSON, AND G. POWER. 1980. Estuarine fish communities of the eastern James – Hudson Bay coast. Environ. Biol. Fishes 5: 135–141.

NEWSHOLME, E. A., V. A. ZAMMIT, AND B. CRABTREE. 1978. The role of glucose and glycogen as fuels for muscle. Biochem. Soc. Trans. 6: 512–520.

PATTERSON, S., I. A. JOHNSTON, AND G. GOLDSPINK. 1974. The effect of starvation on the chemical composition of red and white muscles in the plaice (*Pleuronectes platessa*). Experientia 30: 892–893.

PRIEDE, I. G. 1977. Natural selection for energetic efficiency and relationship between activity level and mortality. Nature (Lond.) 267: 610–611.

ROME, L. C., P. T. LOUGHNA, AND G. GOLDSPINK. 1984. Muscle fiber activity in carp as a function of swimming speed and muscle temperature. Am. J. Physiol. 247: R272–R279.

1985. Temperature acclimation: improved sustained swimming performance in carp at low temperatures. Science (Wash., DC) 228: 194–196.

SIDELL, B. D. 1980. Responses of goldfish (*Carassius auratus* L.) muscle to acclimation temperature: alterations in biochemistry and proportions of different fiber types. Physiol. Zool. 53: 98–107.

SULLIVAN, K. M., AND G. N. SOMERO. 1983. Size- and diet-related variations in enzymic activity and tissue composition in the sablefish, *Anoploma fimbria*. Biol. Bull. 164: 315–326.

TYLER, S., AND B. D. SIDELL. 1984. Changes in mitochondrial distribution and diffusion distances in muscle of goldfish upon acclimation to warm and cold temperatures. J. Exp. Zool. 232: 1–9.

Characterization of Cloned Mitochondrial DNA from the Teleost *Fundulus heteroclitus* and its Usefulness as an Interspecies Hybridization Probe[1]

Lucia Irene González-Villaseñor, Amanda M. Burkhoff, Víctor Corces, and Dennis A. Powers[2]

McCollum-Pratt Institute and Department of Biology, The Johns Hopkins University, Baltimore, MD 21218, USA

González-Villaseñor, L. I., A. M. Burkhoff, V. Corces, and D. A. Powers. 1986. Characterization of cloned mitochondrial DNA from the teleost *Fundulus heteroclitus* and its usefulness as an interspecies hybridization probe. Can. J. Fish. Aquat. Sci. 43: 1866–1872.

Analysis of mitochondrial DNA endonuclease restriction patterns is a powerful tool for studying related species and variation within species. The ethidium bromide staining technique has limited the number of digestions of mitochondrial DNA per individual. Because ^{32}P-end-labeling also imposes severe limitations, we have resorted to cloning the fish (*Fundulus heteroclitus*) mitochondrial genome in the lambda replacement vector EMBL-3. The clone was used as a radioactive probe via Southern blotting to detect mitochondrial DNA restriction fragments obtained by multiple restriction endonuclease digestions from small amounts of tissue. This technique offers much greater sensitivity than ethidium bromide staining. Moreover, it eliminates the expense and time to obtain highly purified mitochondrial DNA for the ^{32}P-end-labeling procedure. It is also useful when the mtDNA is prepared from frozen tissue which has been a problem with the ^{32}P-end-labeling technique. Because the cloned mitochondrial DNA has a high degree of cross-hybridization with the mitochondrial DNA of certain other fishes, it can be used to probe the mitochondrial DNA restriction patterns of a variety of fish species. However, its usefulness is restricted by the degree of relatedness to the species being cloned.

La cartographie de restriction de l'ADN mitochondrial est un outil très utile dans l'étude des espèces apparentées et des variations intraspécifiques. Avec la coloration au bromure d'éthidium, la quantité d'ADN mitochondrial digérée par individu est limitée. Par ailleurs, comme le marquage terminal au ^{32}P comporte aussi de grands inconvénients, nous avons décidé de cloner le génome mitochondrial du poisson étudié (*Fundulus heteroclitus*) dans le vecteur de remplacement lambda EMBL-3. Le clone ainsi obtenu nous a servi de sonde radioactive pour détecter, par blotting Southern, les fragments de restriction d'ADN mitochondrial produits par digestions multiples au moyen d'endonucléases de restriction provenant de petites quantités de tissus. Cette technique est beaucoup plus précise que la coloration au bromure d'éthidium. En outre, elle permet d'épargner temps et argent, car il n'est pas nécessaire de produire l'ADN mitochondrial hautement purifié que nécessite le marquage terminal au ^{32}P. Elle s'applique aussi à l'ADNmt préparé à partir de tissus congelés, cas où le marquage au ^{32}P a posé des difficultés. Comme l'ADN mitochondrial cloné s'hybride très bien avec l'ADN mitochondrial de certains autres poissons, il peut servir à établir la cartographie de restriction de toute une variété d'espèces. Son application est cependant limitée par le degré de parenté entre l'espèce étudiée et celle qui sert à produire le clone.

Received February 25, 1986
Accepted June 12, 1986
(J8698)

Reçu le 25 février 1986
Accepté le 12 juin 1986

Mitochondrial DNA (mtDNA) analysis of restriction endonuclease digestions has been used as a tool for demonstrating nucleotide sequence diversity and determining evolutionary relationships between and within natural populations of a variety of organisms (Avise et al. 1979, 1984; Ferris et al. 1981, 1983; Lansman et al. 1983a; Spolsky and Uzzell 1984; Yonikawa et al. 1981). The mtDNA is isolated and then digested with several restriction endonucleases, generating various sized fragments. The fragments are separated by agarose gel electrophoresis and visualized with ethidium bromide staining, ^{32}P-end-labeling, or via Southern blotting (for procedures see Maniatis et al. 1982).

While the use of mtDNA analysis has profound implications for the study of natural populations, its use has been limited due to the time and expense involved in isolating mtDNA from numerous individuals and the assorted limitations imposed by the various visualization methods. Recently, Chapman and Powers (1984) developed a rapid isolation procedure that allows one to isolate a dozen or more mtDNA samples simultaneously in 6–8 h. Moreover, the procedure is readily applicable to frozen samples and it is much less expensive and time consuming than the more laborious protocols that involve extensive ultracentrifugation.

Visualization of mtDNA fragments with ethidium bromide

[1]Contribution No. 1319 from the McCollum-Pratt Institute and Department of Biology, The Johns Hopkins University.

[2]Author to whom correspondence should be addressed.

staining has severe limitations when the amount of mtDNA available from an individual is small and/or a large number of different restriction digests per sample is desired. While ^{32}P-end-labeling can be used to detect small quantities of mtDNA, each restriction digest must be labeled separately and the mtDNA must be highly purified. The transfer technique originally described by Southern (1975) allows the detection of restriction fragments from small quantities of mtDNA after hybridization with a radiolabeled probe. Unlike ^{32}P-end-labeling, it does not require highly purified mtDNA because contaminating genomic DNA and cytoplasmic nucleic acids (tRNAs) do not cross react with the specific mtDNA radiolabeled probe. The ^{32}P-end-labeling procedure cannot discriminate between mitochondrial and genomic DNA. This latter point may explain why ^{32}P-end-labeling has been of limited value when applied to frozen samples.

To use the Southern method, one needs a mtDNA probe. Such a probe when coupled to a rapid isolation procedure, such as that of Chapman and Powers (1984), allows one to study several populations with large sample sizes, a severe shortcoming of most studies.

Our aim is to determine the evolutionary relationships between and within natural populations of teleosts by analyzing the restriction electromorphs generated by numerous restriction endonucleases from each of several individuals. To accomplish this goal a large number of samples must be collected in the field and processed in the laboratory. Most often, such samples must be frozen. Since several teleost species are small (e.g. *Fundulus heteroclitus* with an average liver weight of 0.1–0.2 g) or because tissue biopsies of commercially important species must be utilized, the amount of mtDNA that can be isolated is often inadequate for ethidium bromide staining. While ^{32}P-end-labeling would provide adequate sensitivity for visualization, it is grossly inadequate for frozen tissue and samples that contain less than highly purified mtDNA. For these and the other reasons alluded to above, the Southern blotting procedure coupled with a rapid isolation procedure has a significant advantage in such studies. Because we are engaged in a detailed analysis of *Fundulus heteroclitus* population dynamics and evolution, this fish's mitochondrial genome was cloned into the lambda replacement vector EMBL-3 and is being used as a hybridization probe. We feel that this approach may act as a model for similar studies in other fish species.

Herein, we describe the procedures for mtDNA isolation, cloning, hybridization, the preparation of the radiolabeled probe, the characterization of cloned mtDNA, and the potential use of the clone as a hybridization probe for studying mtDNA restriction length polymorphisms of natural populations of *Fundulus heteroclitus*, as well as other fish species of scientific and commercial value.

Materials and Methods

Chemicals

Ultrapure agarose, cesium chloride, phenol, restriction endonucleases, bovine serum albumin (BSA), DNase, DNA polymerase I, RNase, nucleotides, bacteriophage lambda, and T4 DNA ligase were from Bethesda Research Laboratories; packagene extract was from Promega Biotec; nitrocellulose membranes were from Schleicher and Schuell, Inc.; deoxycytidine 5′-triphosphate, tetra (triethylammonium) salt (alpha-^{32}P), was purchased from New England Nuclear; ultrapure formamide was obtained from Fluka, AG; Sephadex G-50 medium was from Pharmacia; Tris and ethidium bromide were from Sigma Chemical Co.; all other chemicals were reagent grade.

Fish Tissue

Livers and ovaries were excised from adult fish and either stored in liquid nitrogen until used or processed immediately.

mtDNA Isolation

Many mtDNA isolation procedures have been tried by our laboratory. The fastest, easiest, and most useful for frozen tissues was a slight modification of the method of Chapman and Powers (1984). Although this procedure implies the use of fresh tissues, it works well with tissue that has been frozen and stored for long periods in liquid nitrogen. mtDNA degradation or nicking is eliminated if thawing and refreezing of tissue is avoided. This procedure is advantageous because it does not require the time-consuming CsCl–ethidium bromide ultracentrifugation step necessary for highly purified preparations. In addition, it permits one to process 12 or more individuals simultaneously in one day. Since this method was published in a technical bulletin of limited distribution, we describe the main steps routinely used in our laboratory. For a more complete description, see Chapman and Powers (1984).

Frozen liver (usually between 0.1 and 0.2 g) is homogenized in TEK buffer, pH 7.5 (50 m*M* Tris chloride, 10 m*M* EDTA, 1.5% KCl), the homogenate is poured in a conical disposable tube, and 10 mL of 15% sucrose is released to the bottom of the tube to form a sharp boundary. The homogenate is centrifuged at 4000 rpm per 10 min at 4°C. The upper layer is removed, transferred to polypropylene tube, and centrifuged for 45 min at 13 500 rpm at 4°C. The pellet is rinsed with TEK buffer and the centrifugation step repeated. The pellet is resuspended in TEK buffer containing 1% Nonidet and allowed to stand for 30 min over ice. The suspension is transferred to a microfuge tube and centrifuged for 5 min. The supernatant is transferred to a new microfuge tube and phenol extracted (1:1) twice. This is followed by an extraction with chloroform – isoamyl alcohol (24:1) and precipitation of the mtDNA by adding two volumes of 95% ethanol to the supernatant. The sample is kept at freezing for 3 h. Afterward the sample is microfuged for 30 min, the ethanol is decanted, and the tubes dried until the white pellet turns clear. The mtDNA pellet is resuspended in TEK buffer or sterile distilled deionized water. The sample is then refrigerated or immediately digested with restriction enzymes.

Restriction Analysis of Fish mtDNA

The mtDNA from an individual fish was resuspended in sterile distilled water and divided into several aliquots. Final digest volumes were 20 μL, containing liver mtDNA, 2 μL of 10× restriction buffer, 0.2 μg of RNase, and 2–5 units of restriction endonuclease. Incubation conditions were performed according to the supplier. Reactions were stopped by the addition of 2 μL of 10× dye mixture containing 100 m*M* EDTA, 50% glycerol, 1% SDS, and 0.25% bromophenol blue. Restricted mtDNA was either refrigerated or immediately electrophoresed on a 0.7% agarose slab gel at 40 V for 22 h at room temperature. Bacteriophage lambda fragments produced by digestion with HindIII and EcoRI + ClaI were used as markers.

Southern Transfer

The DNA fragments separated by agarose gel electrophoresis were denatured, neutralized, transferred to a nitrocellulose filter, and immobilized by the procedures described by Maniatis et al. (1982) using 3 *M* NaCl and 0.3 *M* sodium citrate (20× SSC) as the transfer buffer for 18 h.

Insertion of Fish mtDNA in EMBL-3

EMBL-3 is a lambda replacement vector with a cloning capacity of 9–23 kilobases (kb). It is derived by modification of Lambda 1059 (Karn et al. 1980). It contains multiple cloning sites (polylinker sequences SalI–BamHI–EcoRI) between the arms and the stuffer region, allowing the insertion of foreign DNA (Karn et al. 1983; Frischauf et al. 1983).

The *Fundulus heteroclitus* mitochondrial genome of approximately 17.2 kb pair was cloned in the EMBL-3 vector. Approximately 1 μg of fish mtDNA and 2 μg of vector were separately digested with 10 units of BamHI for 2 h at 37°C in 20 m*M* Tris buffer, pH 8.0, containing 7 m*M* $MgCl_2$, 100 m*M* NaCl, and 2 m*M* β-mercaptoethanol. Digestions were coprecipitated and the mixture was resuspended in 10 μL of ligation buffer, pH 7.5, containing 0.66 *M* Tris, 50 m*M* $MgCl_2$, 50 m*M* dithiothreitol, and 10 m*M* ATP, and ligated with 2 units (Weiss) of T4 DNA ligase/μg DNA for 16 h at 16°C. The resulting product was packaged by using the packagene extract and the conditions specified by Promega Biotec. Packaged phage were assayed by plating preadsorbed *Escherichia coli* strains (LE392 and NM359) on Bactoagar plates. Recombinant phage clones and their DNA were extracted according to the procedures of Maniatis et al. (1982). The DNA from the recombinant phage clones was digested with BamHI and subjected to electrophoresis on a 0.3% agarose gel to verify the fragment sizes of the vector's left and right arms and the fish mtDNA. A large-scale preparation of cloned mtDNA was performed according to the procedures of Maniatis et al. (1982).

Preparation of the Radiolabeled Probe

^{32}P-labeled DNA with a specific activity of at least 10^8 cpm/μg was prepared with ^{32}P-dCTP. A typical reaction contained 1 μg of cloned DNA, 5 μL of a buffer containing 5.8 g NaCl/L, 2 g $MgSO_4 \cdot 7H_2O$/L, 1 *M* Tris chloride, pH 7.5, and 2% gelatin (10× SM buffer) (Maniatis et al. 1982), 1 nmol of each unlabeled dATP, dGTP, and dTTP, 50 μCi (1.85 MBq) of ^{32}P-dCTP, and sterile distilled water to a final volume of 48 μL. One microlitre of a 1/1000 dilution of DNase (50 μg/mL) and 1 μL of DNA polymerase I was added to the mixture. The incubation was performed at 16°C for 1 h. The reaction was stopped by addition of 5 μL of 0.5 *M* EDTA, pH 8.0. The nick-translated DNA was separated from unincorporated nucleotides by gel chromatography on Sephadex G-50 medium (Maniatis et al. 1982).

Hybridization Procedure

The nitrocellulose filter containing the fish mtDNA restricted fragments was sealed in a plastic bag (Sears, Seal-and-Save). The filter was prehybridized with a high stringency buffer (60 μL/cm^2 filter), containing 0.75 *M* NaCl, 0.075 *M* sodium citrate (5× SSC solution), 50% formamide, 5× Denhardt's solution (Maniatis et al. 1982), 50 m*M* phosphate buffer, pH 6.8, 50 μg/mL phenol-extracted salmon sperm DNA, and sterile distilled water. The buffer was injected by puncturing the bag with a disposable 25-mL syringe and then sealed. Prehybridization was carried out at 40°C for 4 h. After incubation, an aliquot of approximately 2×10^{10} cpm of ^{32}P-labeled probe was denatured in a boiling water bath for 5 min, rapidly cooled in ice water, and injected into the plastic bag with a 1-mL tuberculine-type disposable syringe. The puncture was sealed and the bag was inverted several times to allow for the mixing of the radiolabeled probe. The bag was incubated for 18 h at 40°C. The hybridized filter was washed twice in a 2× SSC solution for 20 min, twice at 68°C in a 2× SSC solution containing 0.1% SDS for 20 min, and twice in a 0.1× SSC solution for 20 min. Filters were dried between two pieces of Whatman filter (3 MM) before autoradiography. Exposure was carried out against Kodak X-ray film type XAR-5 with Cronex Lighting Plus intensifying screens at −85°C for 24–48 h. When two or more filters were processed together, they were hybridized individually (one filter per bag) but washed simultaneously.

mtDNA Restriction Map

The closed-circular mtDNA of *Fundulus heteroclitus* was characterized as to the sites at which each of 18 restriction endonucleases cleave the DNA. The map was constructed by using conventional double-digestion procedures (Lawn et al. 1978; Maniatis et al. 1982).

The cloned mtDNA was used to construct the map because the restriction sites generated by some of the enzymes used in these studies have been mapped in the EMBL-3 vector (Frischauf et al. 1983). Thus, the left and right arms of the vector were used as reference points to locate the correct positions of the mtDNA fragments generated from the insert.

The restriction enzymes EcoRI, BglII, and BamHI were used in the double digestions. EcoRI generates four widely spaced sites in the inserted mtDNA but does not cleave the vector. BglII has several known sites in the left and right vector arms and cleaves once in the inserted mtDNA, approximately half its length. Since BamHI has a restriction site on each of the vector's polylinker sequences, digestion with BamHI generates one piece of approximately 17.2 kb corresponding to the inserted mtDNA and two pieces corresponding to the vector's left and right arms, respectively.

The position of DNA fragments generated by enzymes with unknown sites in the EMBL-3 vector, but cleaving one to three times the inserted DNA, was confirmed by directly digesting the fish mtDNA by the same double-digestion procedure using EcoRI, BglII, and BamHI. The restriction sites mapped on the linear cloned mtDNA were translated to the normal closed-circular topology of the 17.2 ± 0.3 kb fish mtDNA by using the BamHI site as a reference point with position zero (Fig. 1).

Results

mtDNA Restriction Map

The restriction map of the approximately 17.2 ± 0.3 kb pair circular mtDNA of *Fundulus heteroclitus* is shown in Fig. 1. Map positions for the total of 55 sites recognized by 18 restriction endonucleases are shown along the circular mitochondrial genome.

Cloned mtDNA as a Hybridization Probe

Figure 2 shows the autoradiograph of a single fish's mtDNA electromorph patterns obtained by digestion with each of 18

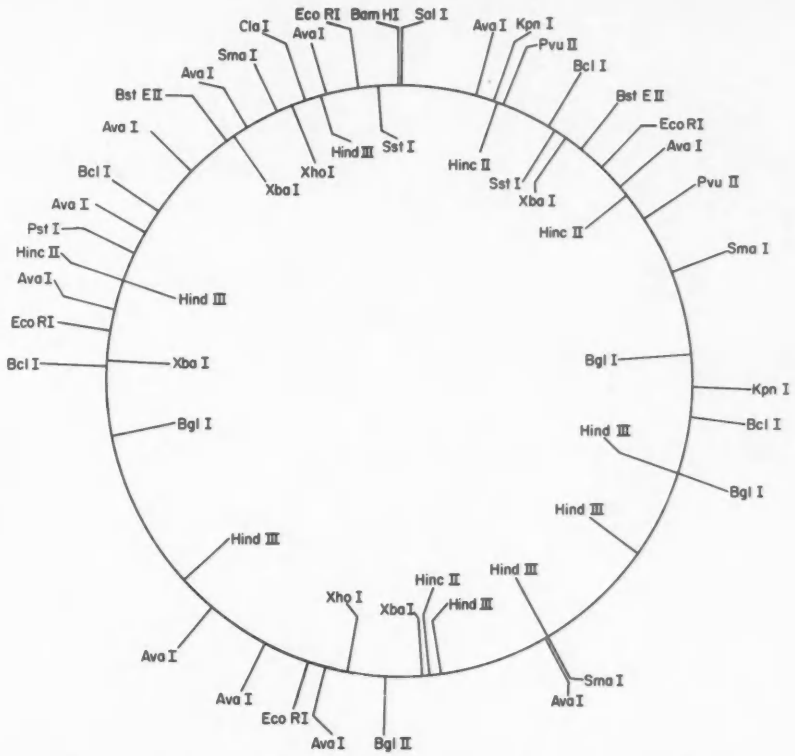


FIG. 1. Restriction map of the 17.2 ± 0.3 kb pair circular mitochondrial DNA of *Fundulus heteroclitus*. Map positions for the total of 55 sites recognized by 18 restriction endonucleases are shown along the circular mitochondrial genome. The mtDNA of *Fundulus* was arbitrarily assigned a total map length of 100 units. The single BamHI restriction site was used as a reference point with position zero. The accuracy of site determination varies by approximately ±300 base pairs. Restriction sites were clockwise mapped.

restriction endonucleases. The visualization of the mtDNA fragments was attained by Southern analysis followed by hybridization with the DNA complementary fragments of the radiolabeled probe containing the cloned mtDNA. This procedure allowed for the detection of small DNA quantities from one individual that otherwise were passed undetected by the conventional ethidium bromide staining procedure.

We have successfully used the clone to survey the mtDNA of two *Fundulus heteroclitus* populations. Measurements of mtDNA divergence have been obtained from the analysis of restriction fragments generated by numerous restriction endonucleases (Powers et al. 1986).

Hybridization of *Fundulus heteroclitus* Cloned mtDNA with Other Fish Species

In a mtDNA survey from various fish species, we found that *Fundulus heteroclitus* cloned mtDNA hybridized with the complementary DNA fragments of the Centrarchidae *Lepomis macrochirus macrochirus, Lepomis macrochirus purpurescens, Lepomis microlophus*, and *Lepomis cyanellus*, the Sciaenidae *Cynoscion regalis*, the Salmonidae *Salmo gairdneri*, the Cichlidae *Tilapia aurea*, the Percichthydae *Morone saxatilis*, and the Ictaluridae *Ictalurus punctatus* (American Fisheries Society 1980). Thus, to determine the degree of hybridization between the *Fundulus* mtDNA and the complementary DNA fragments of each of the various fish species, the agarose gel containing either EcoRI or HindIII restricted mtDNA fragments was stained with ethidium bromide before transferring and hybridization with the radiolabeled probe. The hybridization was carried out at medium stringency conditions (34°C and 30% formamide) to reduce the melting point of the heterologous DNAs. Figure 3 shows both agarose gel and autoradiograph of the HindIII digests. A high degree of cross-hybridization was found between the *Fundulus* mtDNA and the mtDNA of the various fish species tested, suggesting high levels of sequence homology between heterologous DNAs. All the mtDNA restriction electromorphs observed in the agarose gel were also observed in the autoradiograph. However, some bands were lighter, due to a lower homology between the probe and the various fish mtDNAs, indicating a certain degree of difference between these species. Since some bands appeared to have lower homology than others (Fig. 3), one is forced to conclude that the genetic divergence of the mtDNA between *Fundulus heteroclitus* and the species tested is not randomly distributed throughout the mitochondrial genome. Clearly, Fig. 3 indicates that the homology is much greater in some restriction fragments than in others. For routine analysis of a large number of samples of the same species, we recommend either to test for optimum hybridization conditions when using the *Fundulus*

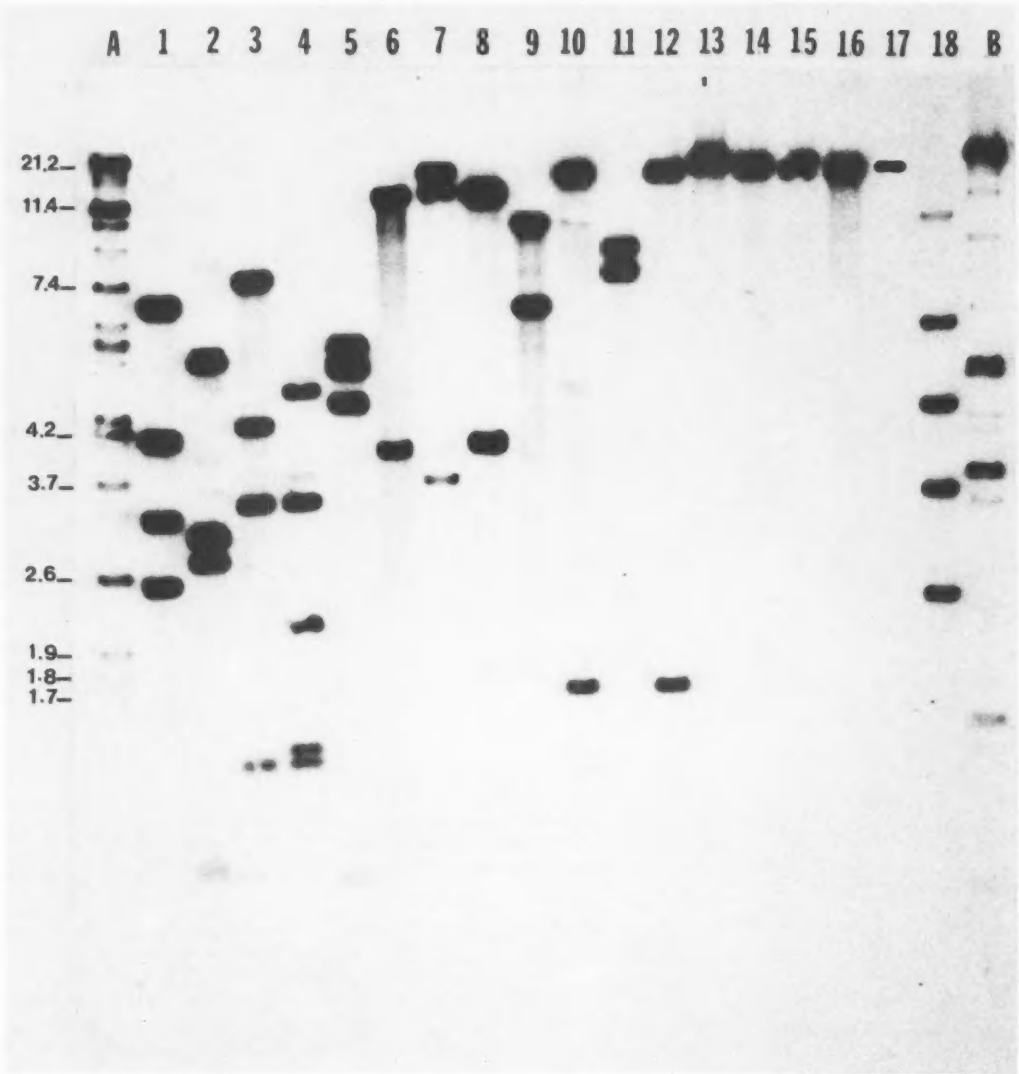


FIG. 2. Autoradiograph of a single fish's mtDNA electromorph patterns generated by digestion with each of 18 restriction endonucleases. The visualization of the mtDNA fragments was attained by Southern analysis followed by hybridization with the complementary DNA of the radiolabeled probe. Bacteriophage lambda fragments produced by digestion with HindIII (A) and EcoRI + ClaI (B) were used as markers. Restriction endonucleases: 1, EcoRI; 2, HindIII; 3, BclI; 4, AvaI; 5, HincII; 6, BstEII; 7, KpnI; 8, Sma; 9, XhoI; 10, SstI; 11, BglI; 12, PvuII; 13, PstI; 14, BamHI; 15, BglII; 16, ClaI; 17, SalI; 18, XbaI.

mtDNA clone or to prepare species-specific clones by the method outlined in this paper.

Discussion

Restriction analysis of mtDNA allows the determination of genetic diversity within and among natural populations. In addition, the mtDNA mode of inheritance (i.e. generally maternally transmitted) (Lansman et al. 1983b) offers a unique opportunity to examine the evolutionary history of female genetic movement and survival in a species, as well as a high-resolution analysis of the evolutionary process and relationships among species. Such studies imply the analysis of an adequate number of individuals of several populations, as well as sufficient quantities of mtDNA to be digested with a reasonable number of restriction enzymes.

Fundulus heteroclitus is an excellent model organism for such studies because it is available in large numbers and populations are relatively continuous along the east coast from Canada to Florida. However, their size limits the amount of available mtDNA. This fact led us to the cloning of their mitochondrial genome to be used as a hybridization probe for the mtDNA restriction analysis of various *Fundulus heteroclitus* populations.

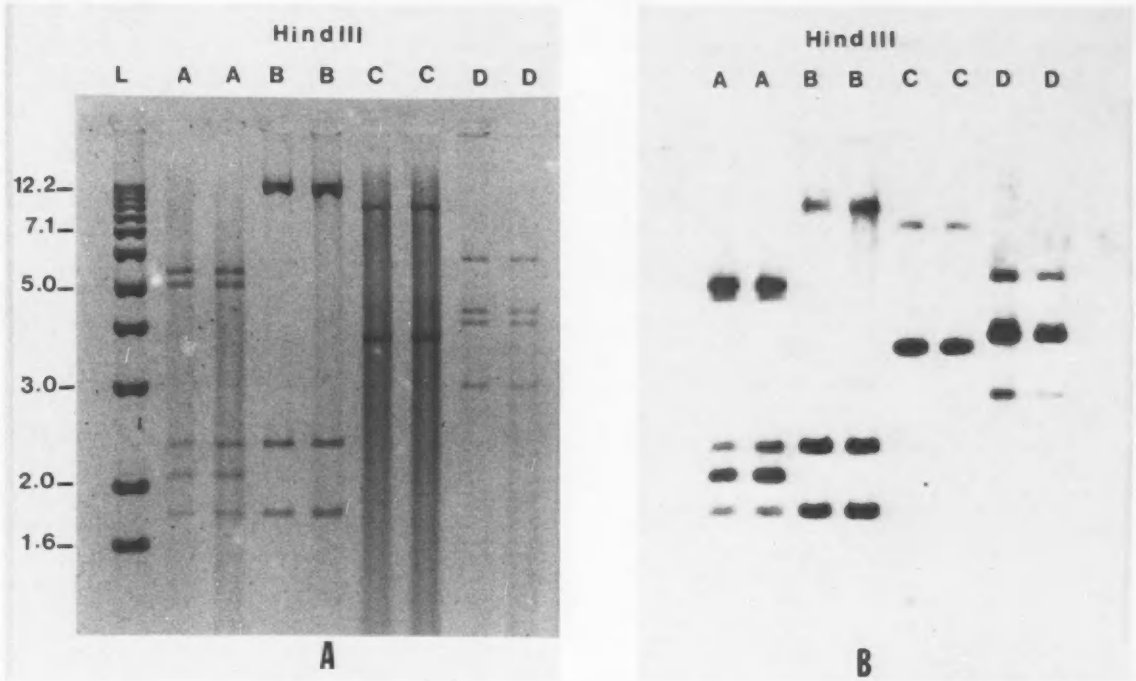


FIG. 3. (A) Ethidium bromide stained 0.8% agarose gel containing HindIII mtDNA digestions of three different fish species. L represents a 1-kb ladder used as a marker. (B) Autoradiograph of the same HindIII digests after Southern analysis followed by hybridization with the DNA complementary fragments of the radiolabeled probe containing the cloned *Fundulus heteroclitus* mtDNA. A A, *Lepomis macrochirus macrochirus*; B B, *Lepomis macrochirus purpurescens*; C C, *Lepomis microlophus*; D D, *Cynoscion nebulosus*.

Although the use of a cloned hybridization probe is necessary for our *Fundulus heteroclitus* studies, it is usually unnecessary for species where large quantities of liver and/or ovary tissue are available. However, even for large fish, cloned mtDNA can be useful if one wishes to keep the fish alive by taking a biopsy, or if one wishes to use only a small piece of tissue from a commercial fishery, thereby not reducing the fish's value. The biopsy may be used with the cloned mtDNA in studies dealing with the evolutionary relationships among various fish species, as well as studies concerned with migration patterns, mating systems, maternal relationships, fisheries stock management, and assessment, etc.

We explored the potential for using a complementary mtDNA probe to identify homologous genes in other fish species by using the Southern blot hybridization. Such analyses demonstrated that *Fundulus heteroclitus* mtDNA has many coding regions homologous with the mitochondrial genome of various fish species. Since the hybridization was carried out under stringent conditions that allowed the reanniling of the probe with the complementary mtDNA of these species, all the fragments observed in the autoradiograph, including the weakly hybridizing fragments, were also observed in the ethidium bromide stained gels. This indicates that the probe can be used in scoring digestion fragments when appropriate hybridization conditions are used. However, as pointed out earlier, Fig. 3 clearly indicates that the degree of homology varies in a nonrandom fashion which is illustrated by the differences in intensity of fragments (Fig. 3B). While these differences are interesting, they do not diminish the usefulness of the probe except for very distantly related species because all the fragments identified by ethidium bromide staining were visible when the heterologous probe was used. The usefulness of this clone relies on (1) its degree of cross-hybridization with the mtDNA of other fish species, (2) its usefulness to detect small mtDNA quantities, and (3) the degree to which the clone has been characterized. Thus, before the clone is used as a heterologous probe for an extensive survey, one must be sure that the patterns obtained are identical in number to those obtained by other visualization methods. If the species being probed is distantly related to the cloned species, some fragments could be eliminated by lack of homology.

The *Fundulus heteroclitus* cloned mtDNA described here is available to anyone upon written request. Moreover, the cloning procedure described herein can be easily adapted to other fish mtDNA to generate species-specific clones, should they be preferred over the heterologous probing procedure alluded to above.

Acknowledgements

We thank Dr. Robert W. Chapman for providing the tissue samples of various fish species used in these studies. We also thank David Muller for his help in all the laboratory procedures and Judith DiMichelle for technical help in the preparation of the mtDNA used for the initial cloning. This work was supported by NSF grant DEB 82-07006 and funds from The Maryland Sea Grant Program.

References

AMERICAN FISHERIES SOCIETY. 1980. A list of common and scientific names of fishes from the United States and Canada. Spec. Publ. No. 12, 4th ed. 174 p.

AVISE, J. C., E. BERMINGHAM, L. G. KESSLER, AND N. C. SAUNDERS. 1984. Characterization of mitochondrial DNA variability in a hybrid swarm between subspecies of bluegill sunfish (*Lepomis macrochirus*). Evolution 38: 931–944.

AVISE, J. C., C. GIBLIN-DAVIDSON, J. LAERM, J. C. PATTON, AND R. A. LANSMAN. 1979. Mitochondrial DNA clones and matriarchal phylogeny within and among geographic populations of the pocket gopher, *Geomys pinetis*. Proc. Natl. Acad. Sci. USA 76: 6694–6698.

CHAPMAN, R. W., AND D. A. POWERS. 1984. A method for the rapid isolation of mitochondrial DNA from fishes. Tech. Rep. No. UM-SG-TS-84-05. Maryland Sea Grant Program, College Park, MD.

FERRIS, S. D., R. D. SAGE, C. M. HUANG, J. T. NIELSEN, U. RITTE, AND A. C. WILSON. 1983. Flow of mitochondrial DNA across a species boundary. Proc. Natl. Acad. Sci. USA 80: 2290–2294.

FERRIS, S. D., A. C. WILSON, AND W. M. BROWN. 1981. Evolutionary tree for apes and humans based on cleavage maps of mitochondrial DNA. Proc. Natl. Acad. Sci. USA 78: 2432–2436.

FRISCHAUF, A-M., H. LEHRACH, A. POUSTKA, AND N. MURRAY. 1983. Lambda replacement vectors carrying polylinker sequences. J. Mol. Biol. 170: 827–842.

KARN, J., S. BRENNER, AND L. BARNETT. 1983. New bacteriophage lambda vectors with positive selection for inserts. Methods Enzymol. 101: 3–19.

KARN, J., S. BRENNER, L. BARNETT, AND G. CESARENI. 1980. Novel bacteriophage lambda cloning vector. Proc. Natl. Acad. Sci. USA 77: 5172–5176.

LANSMAN, R. A., J. C. AVISE, C. F. AQUADRO, J. F. SHAPIRA, AND S. W. DANIEL. 1983a. Extensive genetic variation in mitochondrial DNAs among geographic populations of the deer mouse, *Peromyscus maniculatus*. Evolution 37: 1–16.

LANSMAN, R. A., J. C. AVISE, AND D. HUETTEL. 1983b. Critical experimental test of the possibility of "paternal leakage" of mitochondrial DNA. Proc. Natl. Acad. Sci. USA 80: 1969–1971.

LAWN, R. M., E. F. FRITSCH, R. C. PARKER, G. BLAKE, AND T. MANIATIS. 1978. The isolation and characterization of a linked δ- and β-globin gene from a cloned library of human DNA. Cell 15: 1157–1174.

MANIATIS, T., E. F. FRITSCH, AND J. SAMBROOK. 1982. Molecular cloning. A laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

POWERS, D. A., I. ROPSON, D. C. BROWN, R. VANBENEDEN, R. CASHON, L. I. GONZÁLEZ-VILLASEÑOR, AND J. A. DIMICHELE. 1986. Genetic variation in *Fundulus heteroclitus*: geographic distribution. Am. Zool. 26: 131–144.

SOUTHERN, E. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98: 503–517.

SPOLSKY, C., AND T. UZZELL. 1984. Natural interspecies transfer of mitochondrial DNA in amphibians. Proc. Natl. Acad. Sci. USA 81: 5802–5805.

YONIKAWA, H., K. MORIWAKI, O. GOTOH, J.-I. HAYASHI, J. WATANABE, N. MIYASHITA, M. L. PETRAS, AND Y. TAGASHIRA. 1981. Evolutionary relationships among five subspecies of *Mus musculus* based on restriction enzyme cleavage patterns of mitochondrial DNA. Genetics 98: 801–816.

Methyl Mercury Levels in a Polluted Prairie River–Lake System: Seasonal and Site-Specific Variations, and the Dominant Influence of Trophic Conditions

Togwell A. Jackson[1]

Environment Canada, National Water Research Institute, Western and Northern Region, 501 University Crescent, Winnipeg, Man. R3T 2N6

Jackson, T. A. 1986. Methyl mercury levels in a polluted prairie river–lake system: seasonal and site-specific variations, and the dominant influence of trophic conditions. Can. J. Fish. Aquat. Sci. 43: 1873–1887.

The Moose Jaw River – Qu'Appelle River system of southern Saskatchewan (Canada) and its chain of hypertrophic lakes are polluted with mercury (Hg) and sewage from urban point-sources. Analysis of water, suspended particles, and bottom sediments sampled at different localities at different times of year (from April to November) revealed that methyl mercury (CH_3Hg^+) levels are not controlled by the total abundance of inorganic Hg but are highly dependent on the abundance of organic matter and planktonic algae. CH_3Hg^+ levels were anomalously high in the presence of sewage effluents and associated algal blooms and at times of reduced river discharge, when the flushing and dilution of nutrients were minimal. At elevated organic carbon, nitrogen, and chlorophyll concentrations, CH_3Hg^+ levels were high even if inorganic Hg levels were low; but at low organic carbon, nitrogen, and chlorophyll concentrations, CH_3Hg^+ levels were low even if inorganic Hg levels were high. The results are ascribed principally to stimulation of the activities of Hg-methylating microorganisms by organic nutrients from algae and sewage. The possibility of amerliorating the Hg problem by reducing nutrient pollution is discussed.

Dans le sud de la Saskatchewan (Canada), le réseau hydrographique Moose-Jaw – Qu'Appelle et sa chaîne de lacs hypertrophes sont pollués par le mercure (Hg) et les eaux usées provenant de sources urbaines ponctuelles. L'analyse de l'eau, des particules en suspension et des sédiments du fond recueillis à divers endroits et à différentes époques de l'année (d'avril à novembre) a révélé que les concentrations de méthylmercure (CH_3Hg^+) ne dépendent pas de l'abondance totale du Hg inorganique, mais dépendent beaucoup de l'abondance de la matière organique et des algues planctoniques. Les concentrations de CH_3Hg^+ étaient anormalement hautes en présence d'eaux usées et des inflorescences algales associées, ainsi qu'aux périodes d'étiage, durant lesquelles la prise en charge et la dilution des matières nutritives sont minimales. À des concentrations fortes de carbone organique, d'azote et de chlorophylle, celle du CH_3Hg^+ était élévée même si celle du Hg inorganique était faible. Mais à des concentrations faibles de carbone organique, d'azote et de chlorophylle, celle du CH_3Hg^+ était faible même si celle du Hg inorganique était élevée. Ces résultats sont imputés surtout à l'action des matières organiques présentes dans les algues et les eaux usées, qui stimulent l'activité des microorganismes méthylant le Hg. On considère la possibilité d'atténuer le problème du Hg en réduisant la charge organique.

Received January 31, 1985
Accepted June 17, 1986
(J8093)

Reçu le 31 janvier 1985
Accepté le 17 juin 1986

Inorganic mercury (Hg) introduced as a pollutant into natural waters is mostly scavenged by particulate matter and deposited in bottom sediments. Free Hg^{2+} gradually released from this pool of slightly soluble inorganic Hg is then transformed by microorganisms into the highly bioavailable, toxic, lipophilic species CH_3Hg^+ (methyl mercury) (Jernelöv 1972). The CH_3Hg^+ diffuses into the water column, is taken up by fish and other organisms (either directly from the water or through the food chain), and accumulates in their tissues. Rates of CH_3Hg^+ production and bioaccumulation depend not only on the abundance of inorganic Hg but also on a complex assortment of environmental variables which affect the activities and species composition of the microflora and the availability of the inorganic Hg for methylation. Stimulation of microbial growth by nutrients such as readily metabolized organic matter tends to promote methylation of the available (free bivalent) fraction of the inorganic Hg (Fagerström and Jernelöv 1972; Langley 1973; Furutani and Rudd 1980; Wright and Hamilton 1982; Rudd and Turner 1983; Rudd et al. 1983; Bodaly et al. 1984; Jackson 1984c, 1986), although this tendency is counteracted to a greater or lesser extent by other microbial processes such as the breakdown ("demethylation") of CH_3Hg^+ and the formation of sulfides, which bind Hg, rendering it less available for methylation and bioaccumulation (Fagerström and Jernelöv 1972; Jernelöv 1972; Shariat et al. 1979). The effects of environmental variables are complex and difficult to unravel because Hg transformations are carried out by many different types of microbe that differ in their ecological requirements (Fagerström and Jernelöv 1972; Jernelöv 1972; Shariat et al. 1979; Jackson 1984b, 1986) and because the availability of inorganic Hg for methylation depends on a

[1]Present address: National Hydrology Research Institute, 11 Innovation Blvd., Saskatoon, Sask. S7N 2X8.

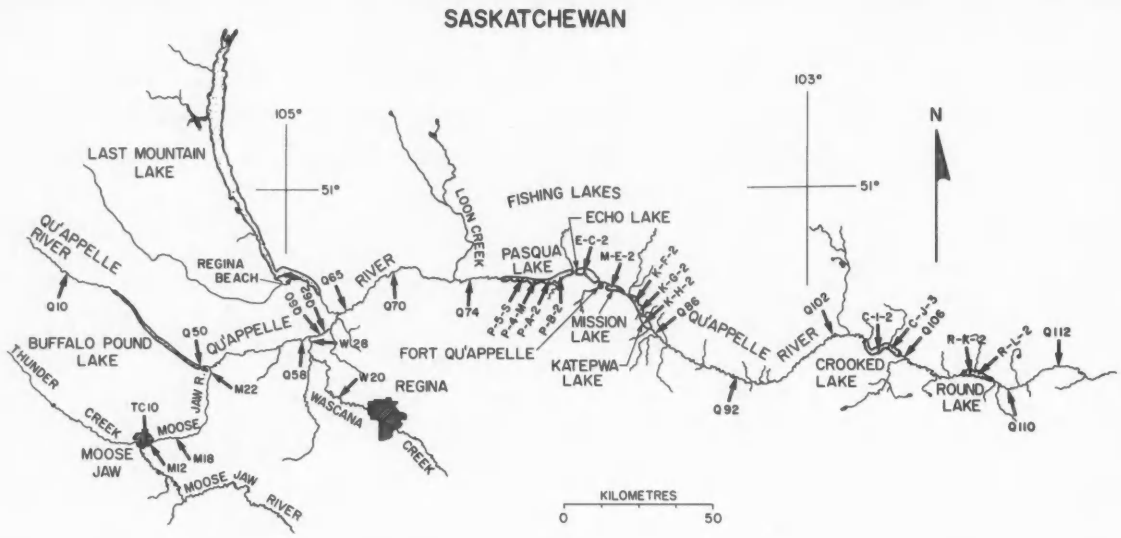


FIG. 1. Map of the field area showing locations of sampling stations (indicated by arrows and letter/number codes).

number of different physicochemical variables.

This report is concerned with the effect of trophic conditions on CH_3Hg^+ levels in the Moose Jaw River – Qu'Appelle River system and associated riverine lakes of southern Saskatchewan, Canada (Fig. 1). The river–lake system has been polluted with Hg, sewage, and other wastes discharged from point-sources in the cities of Moose Jaw and Regina (Gummer and Fast 1979; Gummer 1980; Munro and Gummer 1980; Warwick 1980; Jackson 1983), and one of the riverine lakes (Mission Lake) receives sewage effluents from the town of Fort Qu'Appelle. The Hg is being transported into the river–lake system by two tributaries, Thunder Creek and Wascana Creek. In addition to Hg, toxic pollutants detected in the system include arsenic, lead, cyanide, petroleum products, and various pesticides (Gummer and Fast 1979; Warwick 1980).

This pollution is a cause for serious concern, as the rivers and lakes of the Qu'Appelle River drainage basin comprise an important water resource of the Canadian prairie region (Hammer 1971; Warwick 1980); the lakes are used for a variety of purposes, including commercial and recreational fishing, and adverse effects of Hg contamination on important fish species have already been noted. Hg levels in fish of the Qu'Appelle River lakes commonly exceed 0.5 ppm, the maximum permitted for fish marketed in Canada (Gummer 1980; Jackson 1983), and one result has been that a carp fishery at Pasqua Lake was shut down in 1979. Game fish in these lakes have higher Hg concentrations than fish in unpolluted or less polluted lakes nearby (Buffalo Pound Lake and Last Mountain Lake), the degree of contamination tending to be worst in lakes nearest the sources of pollution (Jackson 1983); thus, there is no doubt about the origin of the Hg. However, little is known about the biogeochemical pathways and cycling of Hg in the river–lake system, and possible synergistic and antagonistic effects of pollutants other than Hg have not, until now, been investigated. Thus, there has been insufficient information on which management decisions for dealing with the problem may be based.

In 1982, I studied forms and biogeochemical pathways of Hg in the Moose Jaw – Qu'Appelle River system in relation to seasonal and geographical variations in environmental conditions, the objective being to assess the Hg problem and suggest possible ways of ameliorating it. This paper deals with selected results of the study together with hitherto unpublished data from research conducted in 1977 and 1978 (also see Jackson 1983, 1984a).

Materials and Methods

Description of Field Area

The field area (Fig. 1) is situated in the Great Plains and has a dry subhumic to semiarid continental climate which supports prairie grassland and transitional parkland vegetation (Hammer 1971; Christiansen et al. 1977). The countryside has been extensively modified by farming and grazing, but native grasses and patches of woodland persist in nonarable uplands, and the floodplain of the Qu'Appelle River supports deciduous trees and marsh communities. The region is covered with a thick blanket of unconsolidated Pleistocene glacial drift and Recent deposits, and generally has a gently undulating topography. The Qu'Appelle Valley is a deep, wide trench bounded by terraces cut by a swollen proglacial river, the ancestor of the present Qu'Appelle River. The lakes of the Qu'Appelle Valley are extremely eutrophic, or "hypertrophic," and typically support dense algal blooms during the growing season (Hammer 1971; Christiansen et al. 1977; Allan and Kenney 1978; Allan and Roy 1980; Warwick 1980). Although these lakes, like many other prairie lakes, are naturally productive — and probably have been since prehistoric times — owing to enrichment with nutrients weathered from glacial drift and concentrated as a result of dry climatic conditions, the process of eutrophication has been accelerated in recent times by additional nutrient loadings due to sewage effluents from cities and towns and runoff from agricultural lands and feed lots in the drainage basin (Christiansen et al. 1977; Allan and Kenney 1978; Warwick 1980).

Sample Collection and Preservation

Field measurements and samples for analysis were taken at 11–14 sites on the Moose Jaw River, the Qu'Appelle River, Thunder Creek, and Wascana Creek (Fig. 1) above and below sources of contamination, and at different distances downstream from these sources, during April 21–26 (when the spring flood was in progress), May 15–19, July 15–21, and November 2–8, 1982. Major basins of the Qu'Appelle River lakes (Fig. 1) were sampled during August 12–18, 1982. Station M12 on the Moose Jaw River just above its confluence with Thunder Creek and station Q50 on the Qu'Appelle River above its confluence with the Moose Jaw River are designated as "control" sites, being situated upstream from the point-sources of Hg pollution at Moose Jaw and Regina (although ponding and backing up of river water during periods of slack flow apparently expose site M12 to occasional contamination); all sites downstream from those two cities are more or less polluted with Hg. Sites just below Moose Jaw, Regina, and Fort Qu'Appelle are contaminated with sewage effluents as well.

Suspended particles in river water were sampled in the field with an Envirodata Sedisamp System-II continuous-flow centrifuge employing a modified disc separator. Operation and recovery characteristics of the centrifuge have been described by Ongley and Blachford (1982). Water samples were taken from rivers and lakes by van Dorn bottle and bottom samples by Ekman dredge. Lacustrine plankton was collected by taking an integrated sample of the water column, from the surface to twice the depth of Secchi disc transparency, with a flexible plastic tube and passing the water (whose volume was recorded) through a 10-μm-mesh net.

Bottom sediments and suspended particles were collected in plastic bags, from which air was then excluded. After brief temporary storage at 4°C, sediment pH and E_h were measured, and all samples of sediment and particulate matter were frozen or freeze-dried and reserved for chemical analysis. Frozen subsamples were used for CH_3Hg^+ and sulfide (S^{2-}) analyses, and freeze-dried subsamples were used for all other analyses. Plankton samples were preserved in Lugol's iodine solution for taxonomic identification and estimation of biomass.

Water samples were divided into filtered and unfiltered aliquots and nonfilterable suspended fractions for determination of "dissolved" and "particulate" constituents and overall water composition. Filtration was performed with 0.45-μm membrane filters and 1.2-μm glass fiber filters (Whatman GF/C) (see below). Aliquots were treated with chemical preservatives or were simply refrigerated, depending on the requirements of the analyses to be performed. CH_3Hg^+ was preserved with H_2SO_4, total Hg with HNO_3 + $K_2Cr_2O_7$, alkali and alkaline earth metals with HCl, and S^{2-} with zinc acetate + glacial acetic acid. Chlorophyll was harvested on GF/C filters on the day of collection and immediately frozen. The supernate from the field centrifuge was not analyzed because the efficiency of the separation between supernate and spun-down solids is seldom perfect and varies with the nature of the suspended solids (Ongley and Blachford 1982) and because contamination from rubber tubes used for conducting water into and out of the centrifuge was considered a possibility.

Analytical Methods

Water

The temperature and conductivity of the water were measured with a YSI 33 meter and the pH with a Corning 610A meter. Dissolved oxygen (O_2) was determined by treatment of sample water with $MnSO_4$ and alkaline iodide/azide solutions, followed by dissolution of the resulting $MnO(OH)_2$ precipitate in H_2SO_4, oxidation of the I^- to I^0, and titration of the I^0 with $Na_2S_2O_3$ solution using starch as an indicator (Stainton et al. 1977). Biochemical oxygen demand (BOD) was estimated as loss of O_2 on incubation in the dark under standardized conditions for 5 d. Total alkalinity was determined by titration with 0.01 *M* H_2SO_4 to the phenolphthalein and bromcresol green/methyl red end points (American Public Health Association et al. 1971). S^{2-} was reacted with *p*-phenylenediamine dihydrochloride and $FeCl_3$ to form a coloured complex (Lauth's violet), whose absorbance at 600 nm was then measured with a Bausch and Lomb Spectronic 100 spectrophotometer for computation of S^{2-} concentration (Stainton et al. 1977).

Water samples to be analyzed for total Hg were digested with $H_2SO_4/KMnO_4/K_2S_2O_8$ at 95°C, after which the dissolved Hg^{2+} was reduced to Hg^0 with $SnSO_4$. The Hg^0 was stripped from solution by sparging with N_2 gas, concentrated on Ag gauze, vapourized by heating, and determined by flameless atomic absorption spectrophotometry (AAS) performed with a Perkin-Elmer 403 unit (Mercury Sampling and Analysis Review Committee 1977). The CH_3Hg^+ fraction of the water was extracted with $CuSO_4$ and $NaBr/H_2SO_4$ solutions and benzene, and following purification of the extracts by $Na_2S_2O_3$ and KI treatments and accompanying polar/nonpolar solvent extractions, the CH_3Hg^+ was determined by gas chromatography (GC) using a Tracor Micro-Tek (MT) 220 GC unit with a Varian electron-capture detector (Uthe et al. 1972). "Inorganic Hg" is here *defined* as the nonmethyl Hg fraction and was *calculated* as total Hg minus CH_3Hg^+. Both filtered and unfiltered water were analyzed for total Hg, but the CH_3Hg^+ content of filtered water could not be determined because it was not feasible to filter the large volumes of water (5 L/sample) required for analysis.

Water samples were analyzed for total organic carbon (C), nitrogen (N), phosphorus (P), and NO_3^- + NO_2^- by the following procedures (Environment Canada 1979, 1984). "Particulate" fractions were collected by passing aliquots of the water through preignited GF/C filters, and "dissolved" fractions were isolated from a second set of aliquots by means of 0.45-μm membrane filters. Particulate organic C and N retained by filters were rinsed with H_2SO_4 to remove inorganic C and were then determined with a Hewlett-Packard 185 CHN analyzer. Filtrates containing dissolved organic C and N, having been acidified and stripped of inorganic C in the form of CO_2, were digested by ultraviolet photooxidation to convert organic C and N to CO_2, NO_3^-, and NO_2^-; the CO_2 was then estimated with an infrared analyzer, and the NO_3^- and NO_2^- were reacted with Cd filings to reduce NO_3^- to NO_2^-, whereupon the NO_2^- was converted to an azo dye by treatment with sulphanilamide and *N*-(1-naphthyl)ethylenediamine dihydrochloride solutions and measured colorimetrically at 550 nm. The dissolved NO_3^- + NO_2^- content of undigested water was recorded as well, and the organic C content of unfiltered water was determined by Beckman 915 total carbon analyzer. Water samples were also analyzed for total and dissolved P by autoclaving with $K_2S_2O_8/H_2SO_4$ solution followed by treatment with H_2SO_4, $(NH_4)_6Mo_7O_{24}$, potassium antimonyl tartrate, and ascorbic acid and spectrophotometric measurement of the resulting blue complex at 880 nm. Dissolved (<0.45 μm) alkali and alkaline earth metals were determined by flame AAS, and

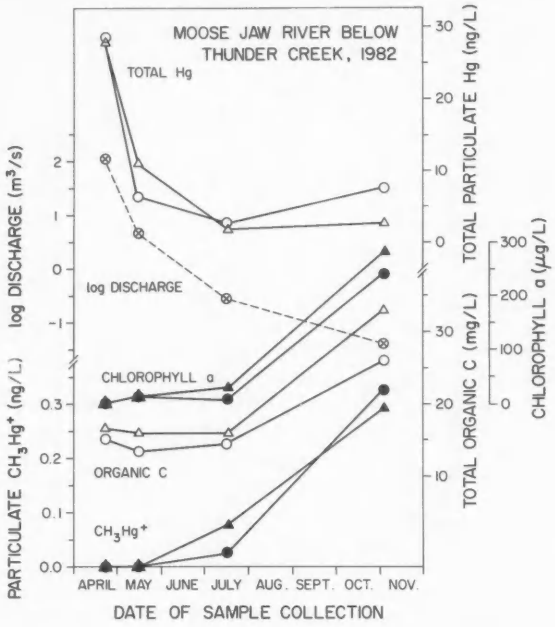


FIG. 2. Seasonal variations in the particulate CH_3Hg^+, total particulate Hg, total (dissolved + particulate) organic C, and chlorophyll *a* concentrations in Moose Jaw River water at sites M18 (○,●) and M22 (△,▲) in 1982, with data for river discharge at site M18 (⊗). (Note: dissolved and particulate organic C fractions were collected by filtration, not centrifugation.)

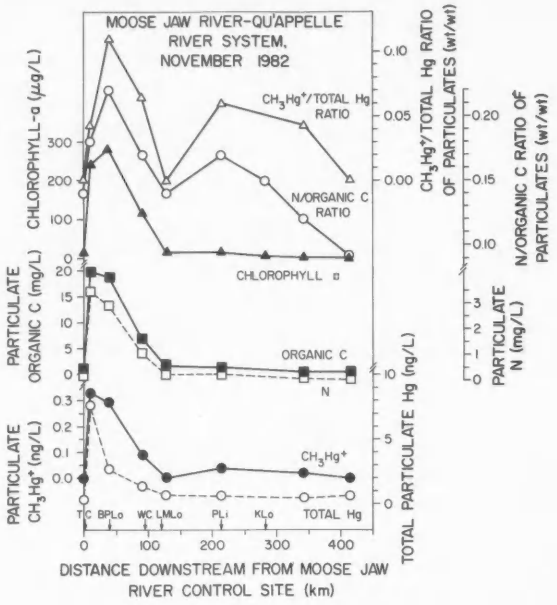


FIG. 3. Variations in the concentrations of total particulate Hg and particulate CH_3Hg^+, organic C, N, and chlorophyll *a* and the CH_3Hg^+/total Hg and N/organic C ratios of suspended particulates in Moose Jaw River and Qu'Appelle River water as functions of distance downstream from the Moose Jaw River control site (M12) in November 1982. (Note: except for the chlorophyll concentrations, all data represent particulate matter collected by centrifugation.) Geographical localities indicated on *x*-axis: TC = confluence of Moose Jaw River and Thunder Creek; BPLo = confluence of Moose Jaw River and ouflow of Buffalo Pound Lake; WC = confluence of Qu'Appelle River and Wascana Creek; LMLo = confluence of Qu'Appelle River and outflow of Last Mountain Lake; PLi = Pasqua Lake inflow; KLo = Katepwa Lake outflow. See Fig. 1.

dissolved Cl^- and SO_4^{2-} were estimated by measurement of conductivity following acidification of an H^+-saturated cation exchange resin before and after removal of Cl^- by precipitation with Ag^+ (Stainton et al. 1977). Chlorophyll *a*, having been collected on GF/C filters from known volumes of water, was extracted with methanol and estimated with a Turner 111 fluorometer (Stainton et al. 1977).

Measurements of the flow rate (discharge) of the river at different sampling sites were furnished by the Water Survey of Canada, which maintains a series of gauge stations along the river system.

Bottom sediments and suspended particles

Sediment pH and E_h were measured with a Corning 12 pH meter (employing platinum and calomel electrodes for the E_h readings). Determination of total Hg, CH_3Hg^+, and inorganic Hg in sediments and suspended particles was accomplished by the same techniques used for water samples (see above), except that CH_3Hg^+ was extracted with toluene instead of benzene, and total Hg was extracted with boiling HCl/HNO_3 (*aqua regia*). Hydrated oxides, or "oxyhydroxides," of iron and manganese (FeOOH and MnOOH) were solubilized by sequential solvent extractions and determined by flame AAS using a Perkin-Elmer 2380 unit. First the readily reducible oxides (mainly MnOOH, with some "amorphous" FeOOH) were extracted with $NH_2OH \cdot HCl/HNO_3$ solution at pH 2; then the less readily reducible oxides (mainly FeOOH, with some MnOOH) were extracted from the undissolved residue with citrate/dithionite solution (Holmgren 1967; Chao 1972; Gupta and Chen 1975). For determination of nonvolatile S^{2-}, the samples were digested with 6 *M* HCl at 60°C to release the S^{2-} as H_2S gas, which was then trapped in zinc acetate solution and estimated by the colorimetric technique employed for the analysis of water. Organic C and N were determined by means of a Carlo-Erba CHN analyzer.

Samples Collected in 1977 and 1978

Dredge samples of surficial bottom sediment were collected from the major basins of the Fishing Lakes chain of the Qu'Appelle River (Fig. 1) on June 29 and 30, 1977, and September 5 and 6, 1978, in the course of two originally unrelated studies. The samples collected in 1977 were analyzed for iron (Fe), while the samples collected in 1978, after measurement of pH and E_h, were analyzed for total Hg, CH_3Hg^+, organic C, N, and S^{2-}. Fe was determined by AAS following digestion with hot $HNO_3/HClO_4$. All other methods used were the same as those outlined above, with the following exceptions: (1) the subsamples analyzed for total Hg were wet-ashed with hot H_2SO_4/HNO_3 (4:1, v/v) instead of *aqua regia*, and they were not freeze-dried prior to digestion; (2) the S^{2-} volatilized as H_2S was trapped in NaOH solution and determined colorimetrically at 228 nm (Armstrong 1975); and (3) all analytical data were computed on the basis of oven-dry (105°C) weight.

Data on chlorophyll concentrations in lake surface water in the summer of 1977 were extracted from the literature (Allan and Roy 1980). The mean chlorophyll concentration for each

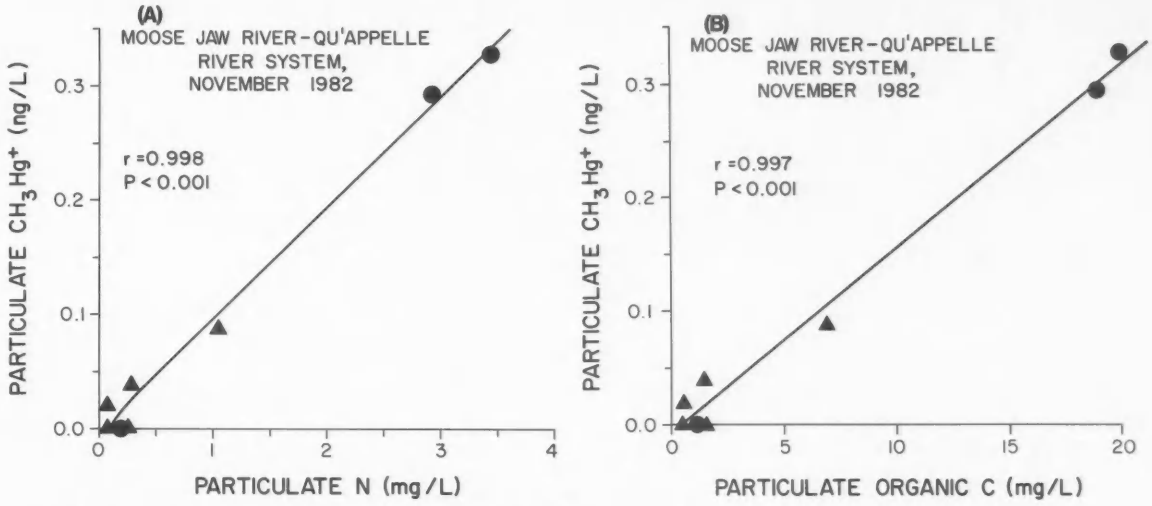


FIG. 4. Relationship between the particulate CH_3Hg^+ and particulate N and organic C concentrations of Moose Jaw River (●) and Qu'Appelle River (▲) water in November 1982. (Note: all data represent particulate matter collected by centrifugation.)

lake was estimated by averaging all data for samples collected at water depths of 0.5 and 1.5 m during July and August.

Results and Interpretations

Suspended Particles in River Water

Seasonal variations in suspended CH_3Hg^+, organic C, and chlorophyll *a* levels of Moose Jaw River water downstream from Moose Jaw were strikingly similar, whereas the concentrations of total suspended Hg, which consisted principally of inorganic Hg,[2] showed a radically different pattern of variation (Fig. 2). CH_3Hg^+, organic C, and chlorophyll levels were low in April, May, and July, but rose sharply to maximum values in early November, when river discharge was lowest and an algal bloom had developed in the virtually stagnant river water. In contrast, the total and inorganic Hg levels were highest in April (during the spring flood), when river discharge was greatest, and were relatively low thereafter, with only a slight rise from July to November. Thus, CH_3Hg^+ concentration was highly dependent on algal biomass and the abundance of organic matter but was essentially independent of inorganic Hg concentration, which was presumably in large part a function of current action and resulting resuspension of Hg-contaminated bottom sediment. Water temperature, despite the decisive influence of temperature on microbial activity, did not have a perceptible effect on CH_3Hg^+ abundance or algal growth within the range of temperatures represented by the samples. In November, when CH_3Hg^+, organic C, and chlorophyll levels were highest, the temperatures were only 1.0−3.3°C, but in the spring and summer, when they were consistently low, the temperatures ranged from 6.6°C (in April) to as high as 18.5°C (in July).

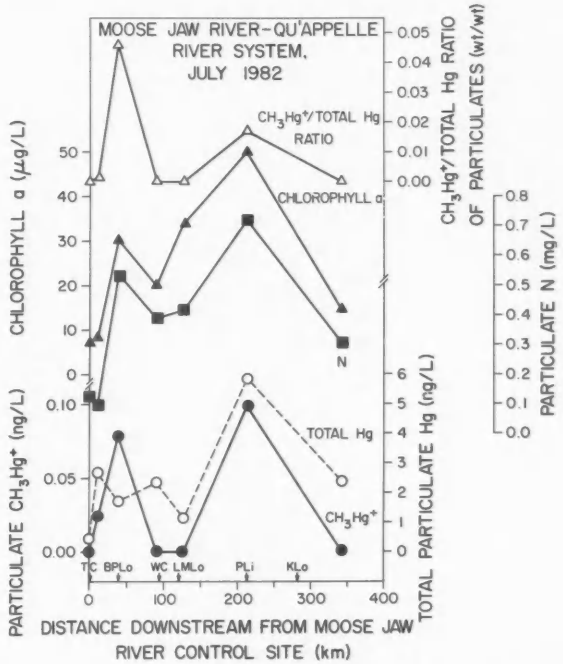


FIG. 5. Variations in the concentrations of total particulate Hg and particulate CH_3Hg^+, N, and chlorophyll *a* and the CH_3Hg^+/total Hg ratio of particulate matter as functions of distance downstream from the Moose Jaw River control site (M12) in July 1982. (Note: except for the chlorophyll concentrations, all data represent particulate matter collected by centrifugation.) See caption to Fig. 3 for geographical localities indicated on *x*-axis.

The study of downstream variations in suspended CH_3Hg^+ levels in the Moose Jaw − Qu'Appelle River system had to be confined to the sample sets for July and November because this

[2] CH_3Hg^+ levels in water, suspended particles, and bottom sediments were consistently at least an order of magnitude lower than inorganic Hg levels. Therefore, total Hg concentration is virtually an estimate of inorganic Hg concentration.

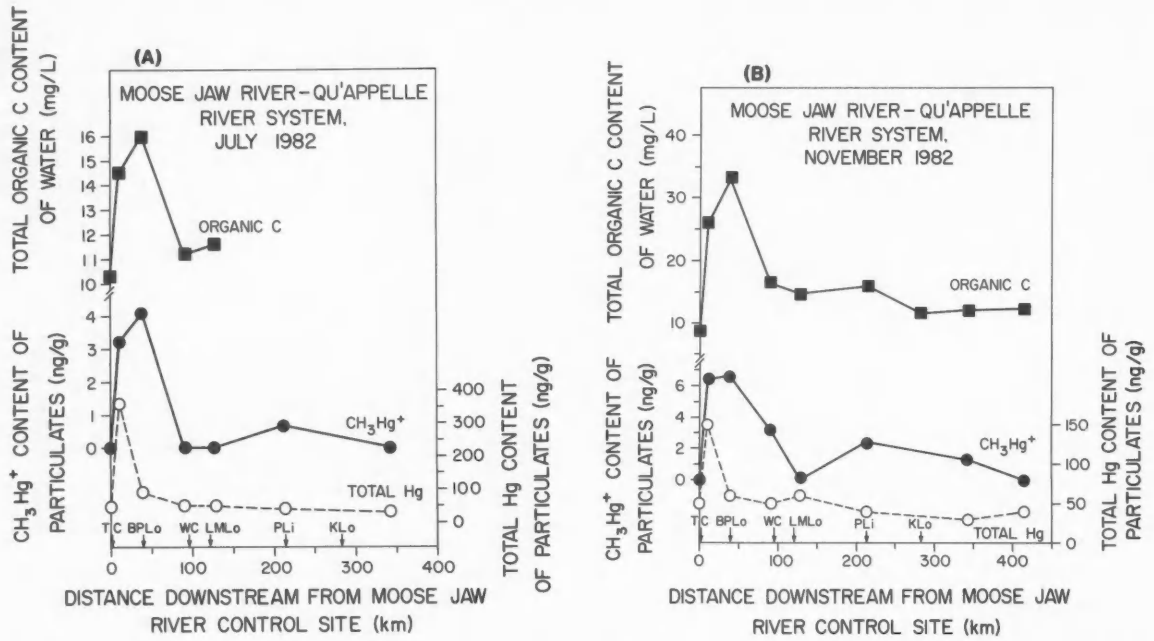

(A)
MOOSE JAW RIVER–QU'APPELLE RIVER SYSTEM, JULY 1982
ORGANIC C
CH_3Hg^+
TOTAL Hg
TC BPLo WC LMLo PLi KLo
TOTAL ORGANIC C CONTENT OF WATER (mg/L)
CH_3Hg^+ CONTENT OF PARTICULATES (ng/g)
TOTAL Hg CONTENT OF PARTICULATES (ng/g)
DISTANCE DOWNSTREAM FROM MOOSE JAW RIVER CONTROL SITE (km)
(B)
MOOSE JAW RIVER–QU'APPELLE RIVER SYSTEM, NOVEMBER 1982
ORGANIC C
CH_3Hg^+
TOTAL Hg
TC BPLo WC LMLo PLi KLo
TOTAL ORGANIC C CONTENT OF WATER (mg/L)
CH_3Hg^+ CONTENT OF PARTICULATES (ng/g)
TOTAL Hg CONTENT OF PARTICULATES (ng/g)
DISTANCE DOWNSTREAM FROM MOOSE JAW RIVER CONTROL SITE (km)

FIG. 6. Variations in the CH_3Hg^+ and total Hg content of suspended particulate matter and the total (dissolved + particulate) organic C content of water from the Moose Jaw River and Qu'Appelle River as functions of distance downstream from the Moose Jaw River control site (M12) in July and November 1982. (Note: dissolved and particulate organic C fractions were collected by filtration, not centrifugation.) See caption to Fig. 3 for geographical localities indicated on *x*-axis.

fraction of the CH_3Hg^+ load in river water was generally detectable only during those months. In April and May, suspended CH_3Hg^+ was detectable only in one or both of the highly polluted tributaries Thunder Creek and Wascana Creek and at one Qu'Appelle River site near the inflow to Pasqua Lake.

Figure 3 shows concentrations of different particulate components of the November samples of Moose Jaw River and Qu'Appelle River water plotted against distance downstream from the Moose Jaw River control site. Again, CH_3Hg^+ correlates more strongly with N ($r = 0.998$), organic C ($r = 0.997$), and chlorophyll ($r = 0.974$) than with inorganic Hg ($r = 0.938$), although all four correlations are highly significant ($P < 0.001$). The relationships of CH_3Hg^+ with N and organic C, which gave the most significant correlations, are shown in Fig. 4. Values for all of these parameters, as expected, rise sharply from the control site to the first polluted Moose Jaw River site, attain their maximum levels near the sources of Hg and municipal sewage, and then decrease with increasing distance downstream therefrom, presumably owing to dilution. However, total Hg declines and tails off much more sharply than CH_3Hg^+, N, organic C, and chlorophyll with increasing distance in the downstream direction. Thus, CH_3Hg^+ levels remain high in the lower Moose Jaw River — apparently because the algal biomass and concentrations of organic matter remain high — despite an approximately two thirds drop in the inorganic Hg concentration. The CH_3Hg^+/total Hg and N/organic C ratios are strongly covariant throughout the sampling region (Fig. 3), indicating that the proportion of CH_3Hg^+ to inorganic Hg is a function of trophic conditions and organic activity.

Dissolved O_2 levels (measured during the day) were high (11.2–16.6 mg/L) throughout the river system in November despite the nearly stagnant conditions and were highest where algal growth was most luxuriant (within 100 km of the Moose Jaw River control site), presumably owing to photosynthesis. Nevertheless, there were detectable S^{2-} concentrations in the water, and anomalously high S^{2-} concentrations (0.089–0.101 mg/L, in contrast with "baseline" values of <0.002–0.022 mg/L), as well as anomalously high S^{2-}/O_2 ratios, were found in the zone of elevated CH_3Hg^+, organic C, N, and chlorophyll concentrations near Moose Jaw, probably reflecting the presence of anaerobic microenvironments surrounding decomposing organic particles (such as dead algae) associated with the algal bloom.

The data for particulate components of river water sampled in July (Fig. 5) are consistent with the results obtained for water sampled in November (Fig. 3), even though the geographic patterns of variation for the two sampling times are different in certain respects. In July, CH_3Hg^+ levels in the Moose Jaw River and upper Qu'Appelle River (within 100 km of the Moose Jaw River control site) corresponded closely with chlorophyll and N — and to a lesser extent with organic C (not shown) — but were independent of inorganic and total Hg levels. CH_3Hg^+, N, chlorophyll, and the CH_3Hg^+/total Hg ratio all peak in the lower Moose Jaw River, just above its confluence with the Qu'Appelle River, whereas there is no total Hg maximum in that region. Further down the Qu'Appelle River (beyond the outflow of Last Mountain Lake), CH_3Hg^+, total Hg, N, and chlorophyll all show a large maximum near the inflow to Pasqua Lake, so that total Hg correlates with CH_3Hg^+; note, however, that these maxima coincide with a

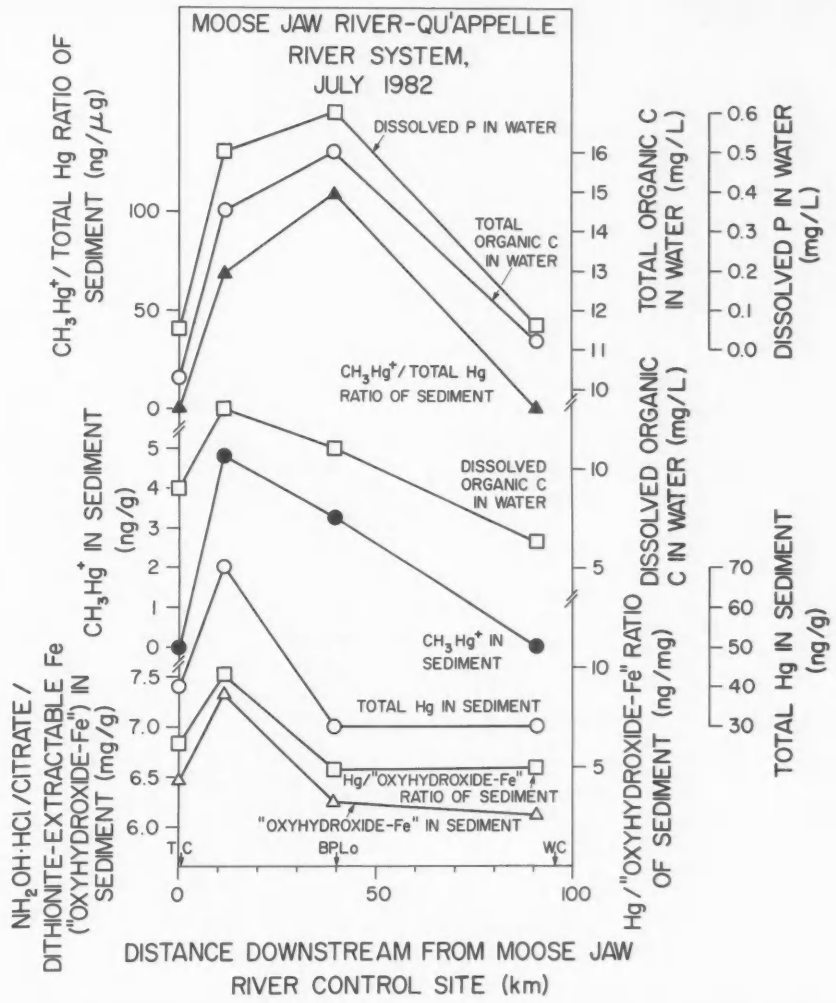

MOOSE JAW RIVER-QU'APPELLE RIVER SYSTEM, JULY 1982
DISSOLVED P IN WATER
TOTAL ORGANIC C IN WATER
CH3Hg+/ TOTAL Hg RATIO OF SEDIMENT
DISSOLVED ORGANIC C IN WATER
CH3Hg+ IN SEDIMENT
TOTAL Hg IN SEDIMENT
Hg/"OXYHYDROXIDE-Fe" RATIO OF SEDIMENT
"OXYHYDROXIDE-Fe" IN SEDIMENT
T.C
BPLo
W.C
CH3Hg+/ TOTAL Hg RATIO OF SEDIMENT (ng/μg)
CH3Hg+ IN SEDIMENT (ng/g)
NH2OH·HCl/CITRATE / DITHIONITE-EXTRACTABLE Fe ("OXYHYDROXIDE-Fe") IN SEDIMENT (mg/g)
TOTAL ORGANIC C IN WATER (mg/L)
DISSOLVED P IN WATER (mg/L)
DISSOLVED ORGANIC C IN WATER (mg/L)
TOTAL Hg IN SEDIMENT (ng/g)
Hg/"OXYHYDROXIDE-Fe" RATIO OF SEDIMENT (ng/mg)
DISTANCE DOWNSTREAM FROM MOOSE JAW RIVER CONTROL SITE (km)

FIG. 7. Variations in the CH_3Hg^+, total Hg, and FeOOH ("oxyhydroxide-Fe") concentrations, the CH_3Hg^+/total Hg and Hg/FeOOH ratios of bottom sediments, and the total (dissolved + particulate) organic C, dissolved organic C, and dissolved P concentrations of water in the Moose Jaw River and Qu'Appelle River as functions of distance downstream from the Moose Jaw River control site (M12) in July 1982. See caption to Fig. 3 for geographical localities indicated on *x*-axis.

peak in the CH_3Hg^+/total Hg ratio, suggesting that the elevated CH_3Hg^+ concentration near Pasqua Lake is due at least in part to nutrient enrichment and enhanced biological productivity, and not to the high inorganic Hg level alone.

Thus far, we have dealt only with concentrations of particulate substances in the water. Computed on this basis (as units of mass per unit volume of water), the data are functions of two different variables: the total concentration of particulate matter in the water and the composition of that material. Now, for the sake of comparison, let us examine downstream variations in the composition of the particulate matter (expressed as units of mass per unit dry weight of suspended solids recovered by centrifugation) and related water chemistry data. The patterns of site-to-site variation in the total Hg and CH_3Hg^+ concentrations in suspended matter and the total organic C content of water were virtually the same in November as in July despite seasonal differences in absolute values (Fig. 6). This demonstrates strikingly the persistence, consistency, and significance of site-specific effects, notably pollution of the Moose Jaw River with Hg and sewage effluents introduced from the city of Moose Jaw. CH_3Hg^+ correlates much more strongly with organic C than with total Hg, further illustrating the overriding importance of nutrient levels and biological activity, and the relative unimportance of variations in the inorganic Hg levels, in regulating the abundance of CH_3Hg^+ in the river system. Total and inorganic Hg peak in the Moose Jaw River just below Moose Jaw but decline abruptly to baseline levels within only 40 km of the source of pollution, while both CH_3Hg^+ and organic C peak downstream from the total Hg maximum and do not return to baseline levels until the water has moved about

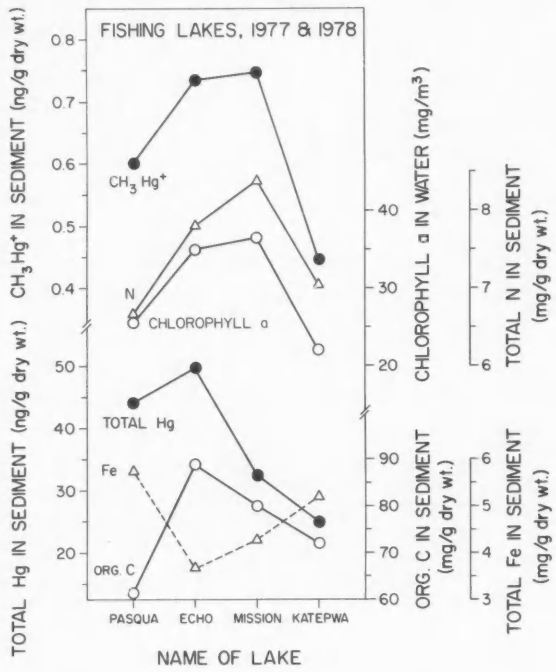


FIG. 8. Variations in the mean CH_3Hg^+, total Hg, organic C, N, and Fe concentrations in bottom sediments and chlorophyll *a* concentrations in near-surface waters of the Fishing Lakes. The chlorophyll and Fe data represent samples collected in the summer of 1977; all other data represent samples collected in the summer of 1978. The chlorophyll data were taken from Allan and Roy (1980).

100 km beyond the source of pollution. Note that a secondary CH_3Hg^+ maximum in the Qu'Appelle River near the inflow of Pasqua Lake coincides with a secondary organic C maximum but is not accompanied by a total or inorganic Hg maximum. (Incidentally, at that particular site the CH_3Hg^+ content of the suspended matter was anomalously high in May as well as in July and November; in May it was the only site in the Moose Jaw – Qu'Appelle River system where detectable suspended CH_3Hg^+ was found.)

In general, the patterns of downstream variation in the suspended CH_3Hg^+ and total suspended Hg concentrations of the water (Fig. 3 and 5) show much less consistency through time than do the patterns shown by the CH_3Hg^+ and total Hg concentrations of the suspended particles (Fig. 6), presumably owing to wide fluctuations in the total suspended load of the water.

Bottom Sediments of Rivers

Downstream variations in the composition of bottom sediments collected in July from the Moose Jaw River and Qu'Appelle River (Fig. 7) were consistent with the results obtained for suspended particles. Below the confluence of Thunder Creek and the Moose Jaw River (the point of entry of Hg into the Moose Jaw River) the concentrations of both total Hg and CH_3Hg^+ rise to maximum values and then taper off, but total Hg drops much more steeply than CH_3Hg^+ does, and therefore reaches background levels much closer to the source of pollution. The pattern of variation shown by CH_3Hg^+ concentration is very similar to that of the dissolved organic C content of the river water, while total Hg is strongly covariant with the FeOOH, or Fe "oxyhydroxide," fraction of the sediment (the sum of all Fe extracted by $NH_2OH \cdot HCl/HNO_3$ and citrate/dithionite treatments) and to a lesser extent with the organic fraction of the sediment as represented by organic C and N (not shown). The CH_3Hg^+/total Hg ratio of the sediment peaks further downstream than either CH_3Hg^+ or total Hg alone and correlates strongly with the total organic C and dissolved P concentrations of the water.

TABLE 1. Correlation between CH_3Hg^+ and nonmethyl Hg in unfiltered water collected from the Moose Jaw River, the Qu'Appelle River, Thunder Creek, and Wascana Creek in April, May, July, and November 1982. N = number of samples; r = correlation coefficient; P = significance probability.

Month of sample collection	N	r	P
April	14	−0.722	>0.001, <0.01
May	14	−0.238	>0.1
July	10	0.533	>0.1
November	13	0.605	>0.02, <0.05

These results further demonstrate that in the presence of high concentrations of major nutrients — in particular, readily metabolized organic matter, which presumably supports intense heterotrophic microbial activity — CH_3Hg^+ levels are high in proportion to the supply of inorganic Hg. Inorganic Hg and CH_3Hg^+ levels to a large extent vary independently of each other and are controlled by quite different processes. The distribution of inorganic Hg appears to be determined by processes such as the adsorption of the Hg by particulate matter rich in FeOOH and subsequent transportation, sorting, and deposition of these carrier particles. As expected, the highest total Hg concentrations and highest ratio of Hg to FeOOH are found just below the point-source of Hg pollution.

Bottom Sediments in Lakes

The sediment samples taken in 1977 and 1978 from the Fishing Lakes (Pasqua, Echo, Mission, and Katepwa) (Fig. 1) gave results leading to the same general conclusions as the sediments and suspended solids collected from the river system in 1982. As demonstrated in Fig. 8, the total Hg levels are highest in the first two lakes of the series (Pasqua and Echo), rising to a maximum in the second lake (Echo) and then declining progressively and markedly in the third and fourth lakes; but the highest CH_3Hg^+ level occurs in the *third* lake (Mission), which receives sewage effluents from the town of Fort Qu'Appelle. The distribution of CH_3Hg^+ in the sediments essentially parallels that of N and is almost identical to the average distribution of chlorophyll in surface water in the previous summer. Again, the results show that CH_3Hg^+ levels are highest where nutrient supplies and biological productivity are greatest — in particular, where sewage effluents and associated algal blooms occur — even if the total supply of inorganic Hg is relatively low. On the other hand, the distribution of total or inorganic Hg, which is similar to that of organic C but inversely related to that of total Fe, appears to be controlled by nonbiological processes such as the transport, sorting, sedimentation, and chemical partitioning of Hg-laden fine-grained fluvial detritus.

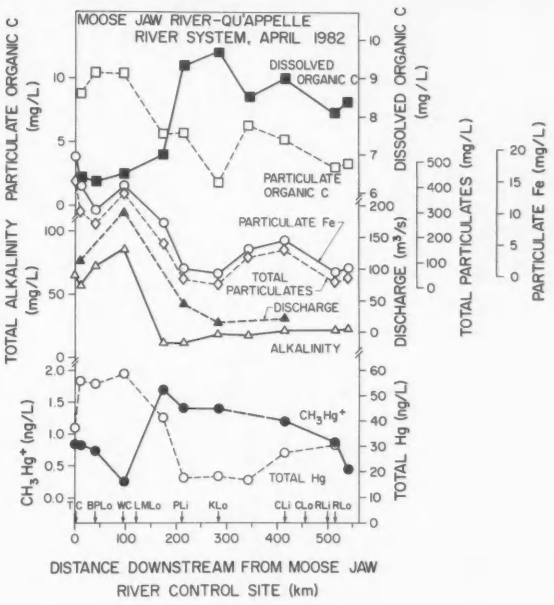


FIG. 9. Variations in the concentrations of CH_3Hg^+, total Hg, dissolved and particulate organic C, particulate Fe, total particulate matter, and the alkalinity and discharge of Moose Jaw River and Qu'Appelle River water as functions of distance downstream from the Moose Jaw River control site (M12) in April 1982. (Note: dissolved and particulate organic C fractions were collected by filtration, but particulate Fe was collected by centrifugation.) See caption to Fig. 3 for geographical localities indicated on x-axis.

River Water

Analysis of whole (unfiltered) water samples from the Moose Jaw River, the Qu'Appelle River, Thunder Creek, and Wascana Creek yielded further evidence that CH_3Hg^+ levels in the river system are regulated primarily by seasonally and geographically varying environmental conditions affecting biological activity rather than by inorganic Hg levels. The data revealed, besides, a striking seasonal trend in the relationship between CH_3Hg^+ and inorganic Hg. All useful information was obtained from unfiltered water. Direct information on dissolved Hg is unavailable because the total Hg data for filtered water had to be discarded owing to evidence that contamination occurred during filtration (the values commonly being higher than values obtained for unfiltered water).

In April (during the spring flood) there was a highly significant *inverse* correlation between CH_3Hg^+ and inorganic Hg, changing to an insignificant inverse correlation in May followed by an insignificant positive correlation in July, and finally a significant, though weak, positive correlation in November (Table 1). Thus, April and November appear to be at opposite extremes, with regular gradations in between. This pattern of seasonal variation parallels the progressive decline in fluvial discharge from April to November (Fig. 2) and is probably linked to it. Possibly these results reflect a decrease in the degree of erosion and resuspension of Hg-contaminated bottom sediment from April to November.

Figure 9 shows downstream variations in the CH_3Hg^+ and total Hg concentrations, and other physicochemical properties, of the river water in April. All curves reveal a distinct discontinuity between 100 and 200 km below the Moose Jaw River control site (M12) because at the time of sampling, most of the Qu'Appelle River water was being diverted into Last Mountain Lake through a creek that normally functions as the lake's outflow, greatly reducing the river's discharge below the point where the river and creek intersect (J. G. Way, Environment Canada, Water Survey of Canada, pers. comm.). Total Hg, particulate Fe, particulate organic C, total particulate load, discharge, and alkalinity all attained their highest levels within 100 km of the control site and then plunged to relatively low levels below the outflow of Last Mountain Lake. It would seem that most of the inorganic Hg was bound to sediment particles eroded and resuspended by current action, and therefore tended to decrease with decreasing discharge. In contrast, CH_3Hg^+ and dissolved organic C levels were low in the rapidly flowing waters within 100 km of the control site but rose sharply to higher levels in the more sluggish waters downstream from the outlet of Last Mountain Lake; consequently, CH_3Hg^+ gave a highly significant inverse correlation with discharge (Fig. 10A). Thus, diminished flushing and dilution in the zone of low flow rates gave rise to elevated concentrations of dissolved nutrients, leading to high concentrations of CH_3Hg^+ despite the accompanying decrease in the pool of total inorganic Hg. Whether there was a concomitant rise in the concentration of dissolved, available forms of inorganic Hg, paralleling the increase in CH_3Hg^+ levels, is not known.

In November, too, the CH_3Hg^+ content of river water gave a highly significant negative correlation with discharge (Fig. 10B), a relationship that is much more significant than the correlation between CH_3Hg^+ and nonmethyl mercury (Table 1). Moreover, the combined Moose Jaw River and Qu'Appelle River water samples for November gave a very strong positive correlation between CH_3Hg^+ and total N (Fig. 10C), as in the case of the particulate CH_3Hg^+ and N fractions of the same samples (Fig. 4). Thus, even when CH_3Hg^+ gave a significant positive correlation with inorganic Hg, it gave a much stronger correlation with factors related to trophic conditions. We may conclude, tentatively, that the effect of nutrient supply and biological activity was the principal factor controlling CH_3Hg^+ abundance.

In November, the CH_3Hg^+, total Hg, N, organic C, and P concentrations all peaked in the Moose Jaw River, near the sources of pollution, and dropped to lower values further down the river system, the patterns of variations closely resembling those shown in Fig. 3.

Lake Water

Surface water from Pasqua Lake, the first body of standing water on the main stem of the river system downstream from the sources of Hg pollution (Fig. 1), not only yielded further evidence for an important link between CH_3Hg^+ abundance and the biomass of planktonic organisms, but also revealed a connection between CH_3Hg^+ and particular planktonic species, besides providing another example of an inverse relationship between the patterns of spatial distribution shown by paired sets of CH_3Hg^+ and total Hg concentrations.

Down the length of Pasqua Lake from the inflow to the outflow (from west to east), the concentration of CH_3Hg^+ and the population sizes of the planktonic blue-green alga *Microcystis wesenbergi* and the planktonic copepod *Cyclops bicuspidatus thomasi* in surface water tended to increase, whereas total Hg and dissolved O_2 concentrations tended to decrease (Fig. 11A).

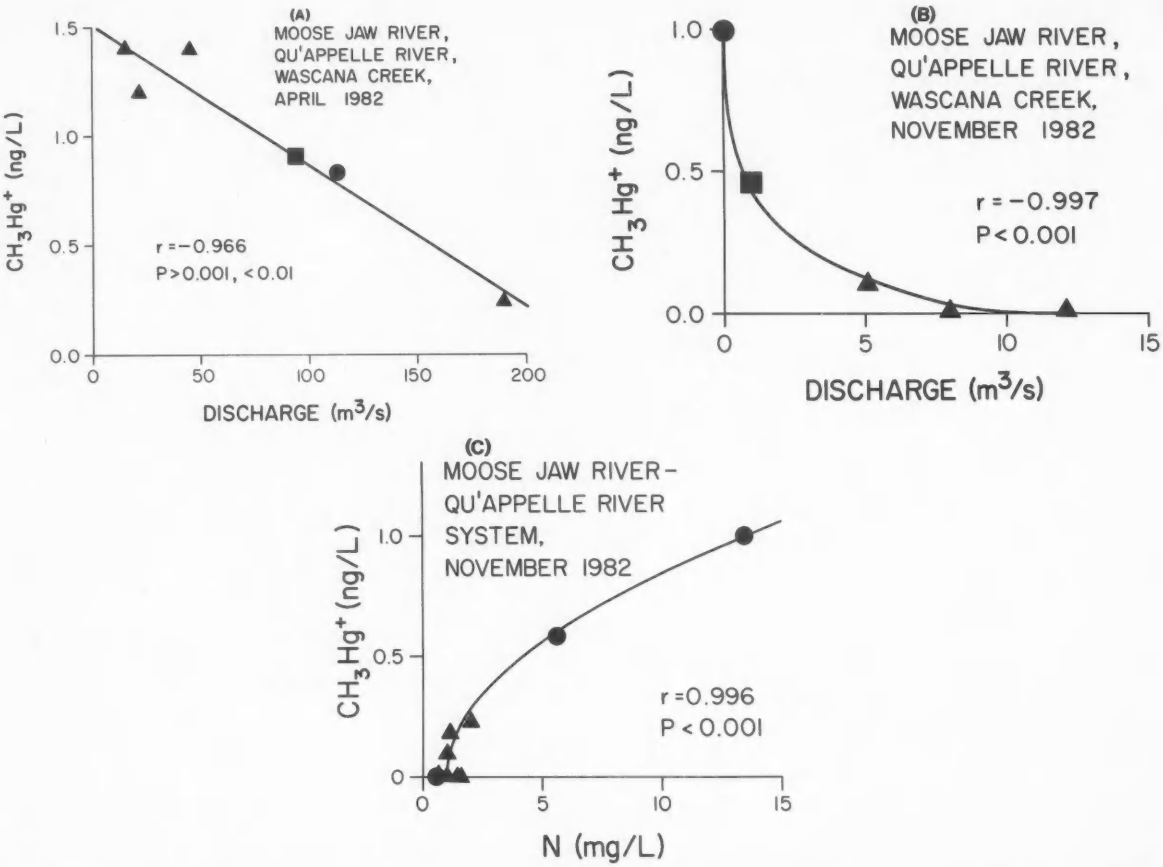


FIG. 10. Relationship between the CH_3Hg^+ content and discharge of Moose Jaw River (●), Qu'Appelle River (▲), and Wascana Creek (■) water in (A) April 1982 and (B) November 1982 and (C) the relationship between CH_3Hg^+ and total N in Moose Jaw River (●) and Qu'Appelle River (▲) water in November 1982.

The CH_3Hg^+ content of the water gave strong positive correlations with the population densities of *M. wesenbergi* and *C. bicuspidatus thomasi*, the significance probability being the same for each ($r = 0.997$; $0.01 > P > 0.001$) (Fig. 11B), even though *M. wesenbergi* comprised only 2% of the phytoplankton biomass and *C. bicuspidatus thomasi* made up only 6% of the total number of planktonic crustaceans. There was also a much weaker but marginally significant inverse relationship between CH_3Hg^+ and dissolved O_2 ($r = -0.951$; $P \approx 0.05$). The combined data for Mission Lake and Katepwa Lake yielded essentially the same relationship between CH_3Hg^+ and dissolved O_2 ($r = -0.990$; $P = 0.01$; no. of samples = 4) except that there was less CH_3Hg^+ per unit quantity of O_2; but Pasqua Lake was the only lake in which the distribution of CH_3Hg^+ corresponded closely with the distribution of particular species of planktonic organisms. Pasqua Lake was also the only Qu'Appelle River lake where *M. wesenbergi* was detected (not counting Echo Lake, which was not sampled), and the mean percentage of blue-green algal biomass in the total phytoplankton biomass was much higher in Pasqua Lake than in the other lakes (80.6% in Pasqua Lake (95.3% excluding the data for a marsh environment near the inflow), but only 3.35, 7.23, 44.7, and 20.4% in Mission Lake, Katepwa Lake, Crooked Lake, and Round Lake, respectively).

Discussion

Considering that CH_3Hg^+ is formed from inorganic Hg and therefore generally exceeds background levels in Hg-polluted aquatic ecosystems (besides sharing with inorganic Hg an affinity for binding agents such as organic and inorganic sulfides), it would not be unreasonable to expect the concentration of CH_3Hg^+ in sediment or water to show a consistent and significant tendency to increase with inorganic Hg concentration. On superficial inspection, this general tendency is indeed discernible, to a greater or lesser extent, in the coarser, larger-scale patterns of variation found in data representing suspended matter and bottom sediments collected at different sites along the Moose Jaw River – Qu'Appelle River – Fishing Lakes system (Fig. 3 and 5–8). Typically, both CH_3Hg^+ and inorganic (or total) Hg concentrations are low in the relatively unpolluted control region, rise to anomalously high values in the polluted region, and then taper off with increasing distance downstream from the point-sources of contamination. More careful and detailed examination of the results, however, leads to the conclusion that the distribution of CH_3Hg^+ in this

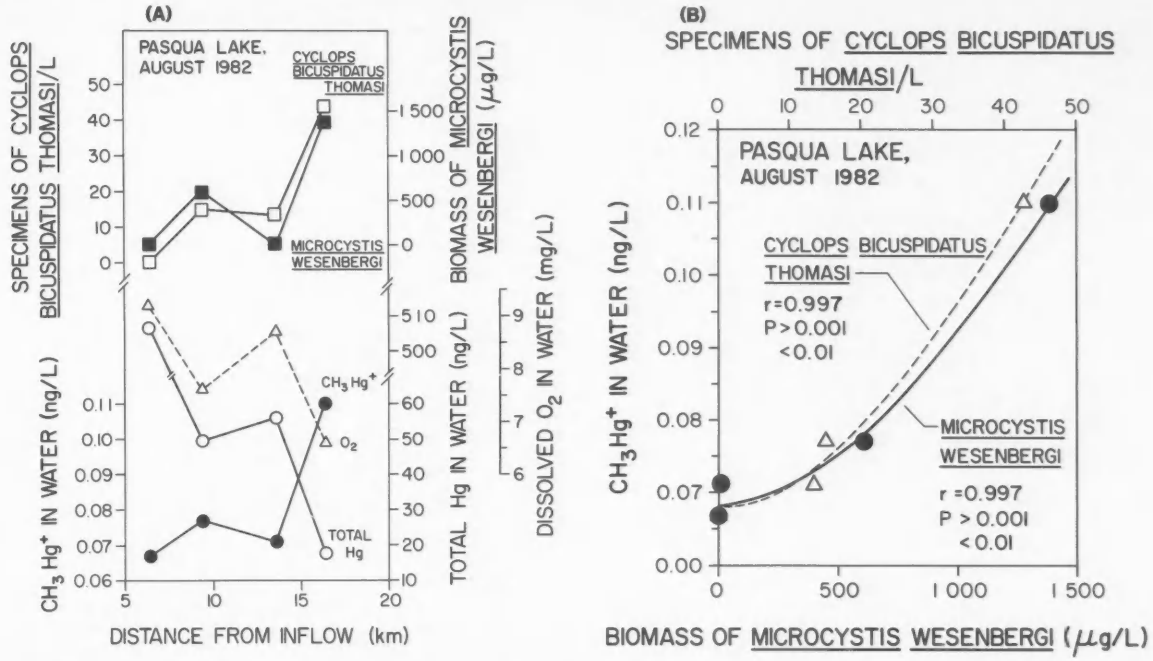


FIG. 11. Variations in (A) the concentrations of CH_3Hg^+, total Hg, and dissolved O_2 and the biomass of *M. wesenbergi* and population density of *C. bicuspidatus thomasi* in Pasqua Lake surface water as functions of distance along a transect from the inflow to the outflow and (B) the relationship between CH_3Hg^+ concentration and the biomass of *M. wesenbergi* (●) and population density of *C. bicuspidatus thomasi* (△) in the water in August 1982.

system is actually controlled by certain physicochemical and biological processes, not by variations in the abundance of inorganic Hg, and that CH_3Hg^+ and inorganic Hg levels respond quite differently to environmental factors, or vary as functions of different sets of environmental parameters. Therefore, CH_3Hg^+ and inorganic Hg levels vary to a large extent independently of each other and may even be inversely related (Table 1; Fig. 2, 9, and 11A).

Probably the reason for the frequent and striking disparities between the seasonal and geographic distribution patterns of CH_3Hg^+ and inorganic Hg is that the efficiency of CH_3Hg^+ production depends not only on the total abundance of inorganic Hg but also on the availability of this Hg for methylation reactions and on the activities of methylating and demethylating microbes, which determine the net rate at which the available fraction of the inorganic Hg is transformed into CH_3Hg^+ (Bisogni and Lawrence 1975; Beijer and Jernelöv 1979). Among the different mutually interconvertible forms of inorganic Hg (Gavis and Ferguson 1972; Jonasson and Boyle 1972; Beneš and Havlík 1979), only free Hg^{2+} is considered directly available for methylation (D'Itri 1972; Gavis and Ferguson 1972; Jernelöv 1972; Wood 1974; Bisogni and Lawrence 1975); consequently, availability is a function of environmental variables such as E_h, O_2 concentration, and concentrations of sulfides, organic matter, hydrated oxides, Cl^-, and other Hg-binding adsorbants and complexing agents which determine the oxidation state, forms, and solubility of the inorganic Hg (Jackson 1979). The activities of Hg-transforming microbes are regulated by environmental factors such as nutrient levels, temperature, E_h, and pH (Fagerström and Jernelöv 1972; Jernelöv 1972; Langley 1973; Bisogni and Lawrence 1975; Shin and Krenkel 1976; Beijer and Jernelöv 1979), and methylation rates correlate with general microbial activity; thus, methylation is generally enhanced by nutrient enrichment and mild temperatures (Fagerström and Jernelöv 1972).

CH_3Hg^+ levels in the sediments, suspended particles, and water of the Hg-polluted Moose Jaw River – Qu'Appelle River – Fishing Lakes system are evidently regulated by trophic conditions and biological productivity, not by inorganic Hg levels, even though particulate and sedimentary CH_3Hg^+ concentrations are admittedly much higher in the severely Hg-polluted regions of the system than in the relatively uncontaminated control regions or (as a rule) at sites far downstream from the point-sources of Hg (Fig. 3 and 5–7). Thus, CH_3Hg^+ gave strong positive correlations with chlorophyll and major nutrients such as organic C and N (Fig. 2–8 and 10), as well as strong negative correlations with the flow rate of the river (presumably owing to increased flushing and dilution of nutrients with increasing flow rate) (Fig. 9 and 10), but commonly failed to correlate significantly with inorganic Hg (Table 1; Fig. 2, 5, 7, and 8), or, surprisingly, gave a significant *inverse* correlation with inorganic Hg (Table 1; Fig. 9 and 11). Even when CH_3Hg^+ showed a significant positive correlation with inorganic Hg, it correlated much more strongly with organic C, N, chlorophyll, and flow rate (Table 1; Fig. 3, 4, and 6). Observations suggesting control of aquatic and sedimentary CH_3Hg^+ levels predominantly by environmental conditions rather than by the supply of inorganic Hg have also been reported for Hg-contaminated river–lake systems of the Canadian Shield (Jackson and Woychuk 1980; Jackson et al. 1982; Jackson 1984b, 1984c, 1986).

The evidence presented here strongly suggests that the sea-

sonal and geographical variations in CH_3Hg^+ abundance in the Moose Jaw River – Qu'Appelle River – Fishing Lakes system are determined primarily by variations in the activities of Hg-transforming heterotrophic microbes, not by variations in the supply of inorganic Hg, and that the principal limiting factor controlling this microbial activity — at least during the ice-free times of year — is the abundance of readily metabolized organic substrates and other major nutrients. According to this interpretation, the close correspondence between the CH_3Hg^+/total Hg ratio of sediment or suspended matter and the N/organic C ratio (Fig. 3) or N and chlorophyll concentrations (Fig. 5) of suspended matter, or the total organic C and dissolved P concentrations of water (Fig. 7), signifies that the efficiency of CH_3Hg^+ production per unit quantity of inorganic Hg depends primarily on the supply of nutrients such as labile nitrogenous organic matter that are available to support the growth of methylating microbes. Stimulation of microbial CH_3Hg^+ production by organic substances may well be the combined effect of utilization of these substances as nutrient substrates by methylating bacteria and exhaustion of O_2 (Fig. 11A) resulting from general heterotrophic microbial activity. A number of workers have reported that net rates of CH_3Hg^+ production are higher under anaerobic conditions than under aerobic conditions, provided sulfide levels are low (Fagerström and Jernelöv 1972; Jernelöv 1972; Olson and Cooper 1976; Jackson 1986). In any case, the relationships between CH_3Hg^+ and major nutrients are entirely consistent with what is known about ecological aspects of microbial methylation of Hg (Fagerström and Jernelöv 1972; Langley 1973; Furutani and Rudd 1980; Wright and Hamilton 1982; Rudd and Turner 1983; Rudd et al. 1983; Bodaly et al. 1984; Jackson 1984c, 1986). On the other hand, considering the important effects of temperature on microbial growth, it is remarkable that CH_3Hg^+ levels were independent of temperature within the rather wide range of temperatures recorded (1.0–18.5°C). Possibly this indicates the presence of Hg-methylating microbes that are adapted to cool conditions and thus are active near the freezing point of water provided there is an ample supply of nutrients (as in the Moose Jaw River in November 1982).

This study has shown that the following environmental conditions, by fostering enrichment of river and lake water with organic nutrients, are particularly favourable for the build-up of large CH_3Hg^+ concentrations in the water and sediments: (1) pollution of the water with sewage effluents (Fig. 3 and 5–8), (2) low flow rates in the river system (Fig. 2, 9, 10A, and 10B), and (3) development of algal blooms (Fig. 2, 3, 5, 8, and 11) owing to high concentrations of nutrients derived from the sewage and other sources. The role of sewage is probably both to furnish organic nutrients for methylating microbes and, by providing N and P, to foster proliferation of algae, whose dead cells and exudates add to the organic substrates available to the microbes.

Although these processes have apparently caused considerable localized enhancement of CH_3Hg^+ production in the vincinity of sewage outfalls, the effects of algal growth on CH_3Hg^+ production are not limited to localities that are heavily polluted with municipal wastes, as illustrated by the data for Pasqua Lake (Fig. 11). Like other inland waters of the prairie region, the river–lake system as a whole is naturally nutrient rich and productive, and there are grounds for believing that dense algal blooms have been endemic in the Qu'Appelle Valley lakes since prehistoric times (Allan and Kenney 1978). Agricultural runoff is also an important, widespread cause of nutrient loading in the region (Christiansen et al. 1977; Warwick 1980).

The close correspondence between CH_3Hg^+ concentration and the biomass of the blue-green algal species *M. wesenbergi* in the surface water of Pasqua Lake (Fig. 11A, 11B) and their inverse relationship with dissolved O_2 (Fig. 11A) suggest that organic nutrients furnished by the recent collapse of an *M. wesenbergi* bloom stimulated the growth of Hg-methylating bacteria in the water column. In general, bacteria readily colonize the gelatinous sheaths of blue-green algal cells, especially in mature algal populations, and their numbers increase greatly when the host cells die, as in the collapse of a bloom (H. J. Kling, Freshwater Institute, Winnipeg, Man., pers. com.). Although *M. wesenbergi* cells accounted for only 2% of the phytoplankton biomass at the time of sampling, they could well have been a surviving remnant of a previously much larger population which had just suffered mass death and was undergoing microbial decomposition accompanied by O_2 consumption, the degree of O_2 exhaustion and the size of the remaining *M. wesenbergi* population both being proportionate to the original biomass of the defunct *M. wesenbergi* bloom. Moreover, *M. wesenbergi* has a thicker gelatinous sheath than *M. aeruginosa* and *Aphanizomenon flos-aquae*, the dominant blue-green algal species in Pasqua Lake at the time of sampling (H. J. Kling, pers. comm.), and may therefore have supported more Hg-methylating bacteria in proportion to its biomass.

The relationship between *C. bicuspidatus thomasi* and CH_3Hg^+ in Pasqua Lake (Fig. 11A, 11B) is probably incidental as far as the factors controlling CH_3Hg^+ production are concerned. There are no grounds for believing that *C. bicuspidatus thomasi* affected microbial growth and Hg methylation to a greater extent than any of the other crustaceans present, as its rate of metabolism and the rate at which it excretes fecal material are not exceptionally high (A. Salki, Freshwater Institute, Winnipeg, Man., pers. comm.). Besides, *C. bicuspidatus thomasi* and CH_3Hg^+, though present in all of the lakes sampled, were covariant only in Pasqua Lake, ruling out any special influence of *C. bicuspidatus thomasi* on CH_3Hg^+ production. Inasmuch as *C. bicuspidatus thomasi* is commonly found in association with phytoplankton communities dominated by blue-green algae because it is less fastidious in its eating habits than its competitors (A. Salki, pers. comm.), its distribution in Pasqua Lake probably reflects a close association with *M. wesenbergi* rather than a role in CH_3Hg^+ production. This is another possible indicator that *M. wesenbergi* had been a dominant member of the phytoplankton community of Pasqua Lake not long before the date of sampling. In brief, it seems reasonable to postulate that both CH_3Hg^+ concentration and *C. bicuspidatus thomasi* population size in Pasqua lake water varied as functions of the biomass of *M. wesenbergi*, though for entirely different reasons.

The variations in the chemical composition of the water and the species composition of the plankton down the length of Pasqua Lake from the inflow to the outflow (Fig. 11A) are of interest because they reflect a well-defined environmental gradient which imposes quite different distribution patterns on CH_3Hg^+ and inorganic Hg. In Pasqua Lake, the depth of water, biological productivity, and proportion of autochthonous organic particulate matter to detrital mineral sediment of fluvial origin increase progressively from the inflow to the outflow (Warwick 1980), and a large delta has formed at the inflow end (Christiansen et al. 1977). The trends depicted in Fig. 11A are

probably related to the distribution of riverborne detritus. Thus, the settling out of suspended detritus resulted in progressive clarification of the water and more effective light penetration, causing progressive enhancement of primary production accompanied by accelerated microbial growth and CH_3Hg^+ production, from the inflow to the outflow. Most of the inorganic Hg was probably bound to suspended fluvial detritus, having been introduced into the lake in that form; therefore, the concentrations of inorganic and total Hg were greatest at the inflow and decreased toward the outflow because of deposition near the mouth of the river as well as progressive dilution with increasing distance away from it. As expected, the distribution of Hg in bottom sediments of the Fishing Lakes (Fig. 8) shows that most of the particulate Hg introduced by the river settles out in the more proximal basins of deposition (in Pasqua Lake and Echo Lake), but it would seem that dilution of this material by relatively coarse, Hg-poor mineral detritus deposited rapidly on the delta at the upstream end of Pasqua Lake and, perhaps, the slow settling rate of finer, more Hg-rich particles have resulted in lower average Hg concentrations in Pasqua Lake than in Echo Lake. There is also evidence for chemical partitioning of inorganic Hg in the lakes. In the river, most of the inorganic Hg is probably bound to detrital FeOOH (Fig. 7 and 9), which may well occur as coatings on detrital mineral grains. The detrital nature of the Fe is reflected in the inverse relationship between Fe and the mainly autochthonous organic C in the sediments of the Fishing Lakes (Fig. 8). The data suggest that after deposition of FeOOH with its adsorbed Hg in the lakes, Hg is released by solubilization of FeOOH in reducing lake-bottom environments and is then taken up by organic matter and sulfides (cf. Fagerström and Jernelöv 1972; Gavis and Ferguson 1972).

The general conclusion that CH_3Hg^+ levels in the river–lake system are regulated primarily by trophic conditions controlling the growth and biochemical activities of Hg methylating and demethylating microbes in the sediments and water column is fully consistent with the results of the study and with current knowledge of the biogeochemistry of Hg. However, this does not exclude the possibility that other factors contributed to the observed effects. One such factor is the degree of availability, or biochemical reactivity, of the inorganic Hg. During the spring flood, Moose Jaw River water had a relatively high particulate inorganic Hg content — presumably owing to erosion and resuspension of Hg-contaminated bottom sediment — but had a low CH_3Hg^+ content (Fig. 2 and 9). The waters of the river system as a whole showed a negative correlation between CH_3Hg^+ and inorganic Hg during the spring flood, giving way to a positive correlation under slack-water conditions (Table 1). Although these results can be explained entirely by variations in trophic conditions and microbial activity, they could also be due at least in part to poor availability of the inorganic Hg bound to resuspended fluvial sediment particles and a relatively high degree of availability under slack-water conditions owing to a higher proportion of the inorganic Hg being in dissolved form (cf. Jackson et al. 1982). Further research would be required to ascertain the relative importance of microbial nutrition and inorganic Hg availability in regulating the abundance of CH_3Hg^+ although the weight of evidence, thus far, appears to favour the conclusion that mirobial nutrition is the principal limiting factor within the range of available inorganic Hg concentrations occurring in this particular river–lake system. Below this range, the abundance of available inorganic Hg could of course become the limiting factor.

Another factor that could have influenced the results is preferential accumulation of CH_3Hg^+ with respect to inorganic Hg by planktonic algae and other organisms. Bioaccumulation of CH_3Hg^+ could help to account for the elevated CH_3Hg^+ concentrations and CH_3Hg^+/total Hg ratios in nutrient-enriched waters containing dense populations of algae. Further research would be needed to test this hypothetical possibility, as CH_3Hg^+ data are not available for the plankton of the river–lake system. It may be mentioned, however, that the total Hg content of phytoplankton in the Qu'Appelle River lakes correlated significantly with the CH_3Hg^+ content of the ambient water ($r = 0.799$; $0.01 > P > 0.001$) but not with the total Hg content of the water ($r = 0.462$; $P > 0.1$), suggesting that the algae accumulated CH_3Hg^+ more efficiently than inorganic Hg (T. A. Jackson, unpub. data). According to the limited available literature on this subject, the CH_3Hg^+/total Hg ratios of marine phytoplankton, though lower than those found in the majority of fish, are much higher than those usually encountered in water and sediments, implying preferential bioaccumulation of CH_3Hg^+ (Knauer and Martin 1972).

Furthermore, physical separation of Hg species owing to differences in their affinities for various nonliving as well as biological metal-binding agents may account, at least in part, for disparities in the seasonal and geographical distribution patterns of CH_3Hg^+ and inorganic Hg (cf. Jackson et al. 1982). The generally steep decline in suspended particulate and sedimentary inorganic Hg levels in the Moose Jaw River with distance downstream from the source of Hg pollution, as contrasted with the more gradual decline in CH_3Hg^+ levels (Fig. 3, 6, and 7), could mean that inorganic Hg was mostly adsorbed to clay and silt, which settled out more readily, causing much of the inorganic Hg mobilized by bottom scour to be redeposited near its source, whereas CH_3Hg^+ was preferentially bound to fine organic particles (including plankton), which had a greater tendency to remain in suspension and move downstream with the current. This interpretation also applies to the high suspended inorganic Hg and low suspended CH_3Hg^+ levels in the river system in April, during the spring flood, as contrasted with the low suspended inorganic Hg and high suspended CH_3Hg^+ levels in July and November (Fig. 2), inasmuch as mineral detritus is resuspended mainly during the spring flood and then largely settles out as current action abates, leaving much of the less rapidly settling fine organic particulate matter in suspension.

Finally, the observed local worsening of the effects of Hg pollution by the discharge of sewage into the river–lake system raises a question which has a bearing on environmental management policy: Would the Hg problem in the river–lake system as a whole be appreciably ameliorated by removal of nutrients from sewage effluents? In Regina, sewage effluents have been undergoing tertiary treatment since 1976 (Kenney 1986), but in Moose Jaw and Fort Qu'Appelle, sewage undergoes primary treatment only (J. G. Way and R. Herrington, Water Survey of Canada, pers. comm.). From the standpoint of suppressing biomethylation of Hg, would anything be gained by introducing secondary and tertiary treatment in Moose Jaw and Fort Qu'Appelle? The answer to this question depends on whether the nutrients in the sewage effluents have an important far-reaching effect on the Qu'Appelle Valley lakes — specifically on accumulation of Hg by lacustrine fish — or merely cause minor, local perturbations. In considering this question, we must take the following facts into account.

(1) Comparison of Hg levels in walleye and pike populations

in different Qu'Appelle Valley lakes for the period 1979–82 has failed to reveal any aberrantly high levels in Mission Lake, the receiving basin for sewage effluents from Fort Qu'Appelle (Jackson 1983). Instead, the total Hg content of the bottom sediments and the influx of Hg-contaminated river water at the inflow of Pasqua Lake have the only obvious effects on Hg accumulation by the fish populations of the lake chain.

(2) Inauguration of tertiary sewage treatment in Regina has, for reasons not yet understood, had no significant effect on the total P influx into Pasqua Lake (Kenney 1986).

(3) The river–lake system is naturally nutrient rich and would undoubtedly continue to nurture algal blooms and intensive microbial activity even if there were no sewage effluents (Allan and Kenney 1978).

For all of these reasons, it seems doubtful whether reduction of municipal waste discharges would significantly ameliorate the Hg problem in this hypertrophic river–lake system. At the most, any improvement would probably occur only in localized regions. However, the subject requires further investigation taking into account the relative importance of pollution from diffuse sources such as agricultural land and feedlots as well as municipal wastes and natural sources.

In any case, the results of the study highlight the fact that the ecological effects of Hg pollution depend not only on the total quantity of Hg involved but also (or perhaps especially) on a number of environmental factors affecting the speciation and bioavailability of the Hg. Hg pollution in an aquatic ecosystem cannot be viewed in isolation from other kinds of pollution occurring simultaneously in the same ecosystem. The possible occurrence of synergistic and antagonistic effects must not be overlooked (Jackson 1984a).

Acknowledgments

Dr. E. D. Ongley helped to plan and direct the research project initiated in 1982, and participated in the field work; in addition, I thank him for reading the manuscript and making useful comments. J. Tisdale and R. N. Woychuk conducted the field program and performed part of the analytical work, and technical assistance was also rendered by C. Baron, P. Kmet, G. Michaud, L. Miller, and L. Peden. Some of the chemical analyses were performed by technicians of the Chemistry Research and Analytical Services section of the Freshwater Institute and the laboratory of the Analytical Services Division of the Water Quality Branch, Environment Canada, in Calgary, Alberta. K. Supeene analyzed the sediments and suspended particulates for CH_3Hg^+. J. G. Way (Water Survey of Canada) kindly furnished data on river discharge and additional information on rivers and sewage effluents in the field area; R. Herrington (Water Survey of Canada) also provided helpful information on sewage. Taxonomic analyses of phytoplankton and zooplankton samples were performed by H. Kling and Dr. A. Salki, respectively. Research performed during 1982–83 was supported by funds awarded under a contract let by the Qu'Appelle Implementation Office, whose financial assistance is gratefully acknowledged. In 1977 and 1978 the field and laboratory operations were largely performed by R. N. Woychuk, with the technical assistance of other workers. Certain chemical analyses were performed by technicians of the Chemistry Research and Analytical Services Section of the Freshwater Institute. Research done in 1977 and 1978 was financed by the author's budget allocation from Environment Canada.

References

ALLAN, R. J., AND B. C. KENNEY. 1978. Rehabilitation of eutrophic Prairie lakes in Canada. Verh. Int. Ver. Limnol. 20: 214–224.

ALLAN, R. J., AND M. ROY. 1980. Lake water nutrient chemistry and chlorophyll *a* in Pasqua, Echo, Mission, Katepwa, Crooked, and Round lakes on the Qu'Appelle River, Saskatchewan. National Water Research Institute, Inland Waters Directorate (Environment Canada) Scientific Series No. 112: 68 p.

AMERICAN PUBLIC HEALTH ASSOCIATION, AMERICAN WATER WORKS ASSOCIATION, AND WATER POLLUTION CONTROL FEDERATION. 1971. Standard methods for the examination of water and wastewater. 13th ed. American Public Health Association, Washington, DC. 874 p.

ARMSTRONG, F. A. J. 1975. Spectrophotometric determination of sulfide in water, p. 14–19. Water Quality Parameters, ASTM STP 573. American Society for Testing and Materials, Philadelphia, PA.

BEIJER, K., AND A. JERNELÖV. 1979. Methylation of mercury in aquatic environments, p. 203–210. *In* J. O. Nriagu. [ed.] The biogeochemistry of mercury in the environment. Elsevier/North Holland Biomedical Press, Amsterdam, New York, Oxford.

BENEŠ, P., AND B. HAVLÍK. 1979. Speciation of mercury in natural waters, p. 175–202. *In* J. O. Nriagu [ed.] The biogeochemistry of mercury in the environment. Elsevier/North Holland Biomedical Press, Amsterdam, New York, Oxford.

Bisogni, J. J., and A. W. Lawrence. 1975. Kinetics of mercury methylation in aerobic and anaerobic aquatic environments. J. Water Pollut. Control Fed. 47: 135–152.

BODALY, R. A., R. E. HECKY, AND R. J. P. FUDGE. 1984. Increase in fish mercury levels in lakes flooded by the Churchill River diverion, northern Manitoba. Can. J. Fish. Aquat. Sci. 41: 682–691.

CHAO, T. T. 1972. Selective dissolution of manganese oxides from soils and sediments with acidified hydroxylamine hydrochloride. Soil Sci. Soc. Am. Proc. 36: 764–768.

CHRISTIANSEN, E. A., D. F. ACTON, R. J. LONG, W. A. MENELEY, AND E. K. SAUER. 1977. Fort Qu'Appelle geolog. Interpretive Report No. 2, Saskatchewan Museum of Natural History, Saskatchewan Culture and Youth. Modern Press, Saskatoon, Sask. 82 p.

D'ITRI, F. M. 1972. The environmental mercury problem. Chemical Rubber Co. Press, Cleveland, OH. 124 p.

ENVIRONMENT CANADA. 1979. Analytical methods manual. Environment Canada, Inland Waters Directorate, Water Quality Branch, Ottawa, Ont.

1984. NAQUADAT — dictionary of parameter codes. Environment Canada, Inland Waters Directorate, Water Quality Branch, Data Systems Sections, Ottawa, Ont.

FAGERSTRÖM, T., AND A. JERNELÖV. 1972. Some aspects of the quantitative ecology of mercury. Water Res. 6: 1193–1202.

FURUTANI, A., AND J. W. M. RUDD. 1980. Measurement of mercury methylation in lake water and sediment samples. Appl. Environ. Microbiol. 40: 770–776.

GAVIS, J., AND J. F. FERGUSON. 1972. The cycling of mercury through the environment. Water Res. 6: 989–1008.

GUMMER, W. D. 1980. Variability and loading of mercury in a small prairie river. Bull. Environ. Contam. Toxicol. 25: 530–536.

GUMMER, W. D., AND D. FAST. 1979. Mercury in the sediments of Thunder Creek and the Moose Jaw River. Report of the Moose Jaw River Basin Working Group on Mercury, Environment Canada (Water Quality Branch) and Government of Saskatchewan (Department of the Environment, Water Quality Division), Regina, Sask. 47 p.

GUPTA, S. K., AND K. Y. CHEN. 1975. Partitioning of trace metals in selective chemical fractions of nearshore sediments. Environ. Lett. 10: 129–158.

HAMMER, U. T. 1971. Limnological studies of the lakes and streams of the upper Qu'Appelle River system, Saskatchewan, Canada. I. Chemical and physical aspects of the lakes and drainage system. Hydrobiologia. 37: 473–507.

HOLMGREN, G. G. S. 1967. A rapid citrate-dithionite extractable iron procedure. Soil Sci. Am. Proc. 31: 210–211.

JACKSON, T. A. 1979. Relationships between the properties of heavy metals and their biogeochemical behaviour in lakes and river–lake systems, p. 457–460. Heavy metals in the environment. Proc. Int. Conf. on Management and Control of Heavy Metals in the Environment, London, Sept. 1979. CEP Consultants Ltd., Edinburgh, Scotland.

1983. Mercury in the Qu'Appelle River system of Saskatchewan and its lakes and tributaries. Report presented to Qu'Appelle Implementation Office, Regina, Sask. 49 p.

1984a. Biogeochemical pathways of mercury in a prairie river–lake system: seasonal and site-specific variations (abstract), p. 728. *In* Environmental contamination. Proc. Int. Conf. on Environmental Contamination, London, July 10–13, 1984. CEP Consultants Ltd., Edinburgh, Scotland.

1984b. Effects of inorganic cadmium, zinc, copper, and mercury on methyl mercury production in polluted lake sediments — evidence for selective inhibition and stimulation of microbial species based on vari-

ations in heavy metal tolerance, p. 551–578. *In* J. O. Nriagu [ed.] Environmental impacts of smelters. Wiley-Interscience (John Wiley & Sons), New York, Toronto, Chichester, Brisbane, Singapore.

1984c. Speciation, mobilization, and biological accumulation of mercury in recently formed reservoirs of northern Manitoba (abstract), p. 45 of Program. 27th Conf. on Great Lakes Research, April 29 – May 3, 1984. International Association for Great Lakes Research, Toronto, Ont.

1986. Methylation, demethylation, and bio-accumulation of mercury in lakes and reservoirs of northern Manitoba, with particular reference to effects of environmental changes caused by the Churchill–Nelson River diversion. Summary Report, Canada–Manitoba Agreement on the Study and Monitoring of Mercury in the Churchill River Diversion. (To be published jointly by the governments of Canada and Manitoba.)

JACKSON, T. A., J. W. PARKS, P. D. JONES, R. N. WOYCHUK, J. A. SUTTON, AND J. D. HOLLINGER. 1982. Dissolved and suspended mercury species in the Wabigoon River (Ontario, Canada): seasonal and regional variations. Hydrobiologia 92: 473–487.

JACKSON, T. A., AND R. N. WOYCHUK. 1980. Mercury speciation and distribution in a polluted river–lake system as related to the problem of lake restoration, p. 93–101. *In* Restoration of lakes and inland waters. Proc. Int. Symp. on Inland Waters and Lake Restoration, Sept. 8–12, 1980, Portland, Maine. U.S. Environmental Protection Agency, Office of Water Regulations and Standards, Washington, DC.

JERNELÖV, A. 1972. Factors in the transformation of mercury to methylmercury, p. 167–172. *In* R. Hartung and B. D. Dinman [ed.] Environmental mercury contamination. Ann Arbor Science Publishers, Ann Arbor, MI.

JONASSON, I. R., AND R. W. BOYLE. 1972. Geochemistry of mercury and origins of natural contamination of the environment. Can. Min. Metal. (CIM) Bull. 65: 32–39.

KENNEY, B. C. 1986. Dynamics of phosphorus in a chain of lakes: the Fishing Lakes. Inland Waters Directorate Scientific Series Report, Environment Canada, Ottawa, Ont. (In press.)

KNAUER, G. A., AND J. H. MARTIN. 1972. Mercury in a marine pelagic food chain. Limnol. Oceanogr. 17: 868–876.

LANGLEY, D. C. 1973. Mercury methylation in an aquatic environment. J. Water Pollut. Control Fed. 45: 44–51.

MERCURY SAMPLING AND ANALYSIS REVIEW COMMITTEE. 1977. Mercury methods for sampling, preservation, and analysis. Environment Canada, Ottawa, Ont. 126 p.

MUNRO, D. J., AND W. D. GUMMER. 1980. Mercury accumulation in biota of Thunder Creek, Saskatchewan. Bull. Environ. Contam. Toxicol. 25: 884–890.

OLSON, B. H., AND R. C. COOPER. 1976. Comparison of aerobic and anaerobic methylation of mercuric chloride by San Francisco Bay sediments. Water Res. 10: 113–116.

ONGLEY, E. D., AND D. P. BLACHFORD. 1982. Application of continuous flow centrifugation to contaminant analysis of suspended sediment in fluvial systems. Environ. Technol. Lett. 3: 219–228.

RUDD, J. W. M., AND M. A. TURNER. 1983. The English–Wabigoon River system: V. Mercury and selenium bioaccumulation as a function of aquatic primary productivity. Can. J. Fish. Aquat. Sci. 40: 2251–2259.

RUDD, J. W. M., M. A. TURNER, A. FURUTANI, A. L. SWICK, AND B. E. TOWNSEND. 1983. The English–Wabigoon River system: I. A synthesis of recent research with a view towards mercury amelioration. Can. J. Fish. Aquat. Sci. 40: 2206–2217.

SHARIAT, M., A. C. ANDERSON, AND J. W. MASON. 1979. Screening of common bacteria capable of demethylation of methylmercuric chloride. Bull. Environ. Contam. Toxicol. 21: 255–261.

SHIN, E.-B., AND P. A. KRENKEL. 1976. Mercury uptake by fish and biomethylation mechanisms. J. Water Pollut. Control Fed. 48: 473–501.

STAINTON, M. P., M. J. CAPEL, AND F. A. J. ARMSTRONG. 1977. The chemical analysis of fresh water. 2nd ed. Fish. Mar. Serv. Misc. Spec. Publ. No. 25. Department of Fisheries and Oceans, Ottawa, Ont. 180 p.

UTHE, J. F., J. SOLOMON, AND B. GRIFT. 1972. Rapid semi-micro method for the determination of methyl mercury in fish tissue. J. Assoc. Off. Anal. Chem. 55: 583–589.

WARWICK, W. F. 1980. Pasqua Lake, southeastern Saskatchewan: a preliminary assessment of trophic status and contamination based on the Chironomidae (Diptera), p. 255–267. *In* D. A. Murray [ed.] Chironomidae: ecology, systematics, cytology, and physiology. Pergamon Press, Oxford and New York.

WOOD, J. M. 1974. Biological cycles for toxic elements in the environment. Science (Wash., DC) 183: 1049–1052.

WRIGHT, D. R., AND R. D. HAMILTON. 1982. Release of methyl mercury from sediments: effects of mercury concentration, low temperature, and nutrient addition. Can. J. Fish. Aquat. Sci. 39: 1459–1466.

Limestone Slurry Reduces Physiological Stress and Increases Survival of Atlantic Salmon (*Salmo salar*) in an Acidic Norwegian River

B. O. Rosseland[1] and O. K. Skogheim[1]

Directorate for Nature and Management, Fish Research Division, Tungasletta 2, N-7000 Trondheim, Norway

H. Abrahamsen

Norsk Biotech A/S, P.O. Box 788, Krossen, N-4301 Sandnes, Norway

and D. Matzow

Miljøvernavdelingen, Fylkeshuset, N-4800, Arendal, Norway

Rosseland, B. O., O. K. Skogheim, H. Abrahamsen, and D. Matzow. 1986. Limestone slurry reduces physiological stress and increases survival of Atlantic salmon (*Salmo salar*) in an acidic Norwegian river. Can. J. Fish. Aquat. Sci. 43: 1888–1893.

Smolts of Atlantic salmon (*Salmo salar*) were exposed for 70 h in the acidic River Vaaraana, southern Norway (Q = 1.3 m^3/s, pH = 4.4–5.6, Ca = 0.6–1.5 mg/L, labile Al = 50–100 μg/L), to waters neutralized by addition of limestone slurry. Chemical and biological parameters were measured upstream of the liming site and 20, 100, 1000, 2000, and 3500 m downstream. Addition of limestone slurry effectively increased pH from 4.6 to 6.9, Ca from 0.6 to 2.6 mg/L, and reduced the labile Al concentration from 59 to 35 μg/L immediately downstream of the liming site (20 m, after 30 s). In the untreated upstream waters, salmon smolts lost plasma Cl rapidly, and all fish died within 36 h. In spite of the slight oversaturation of labile Al immediately downstream of the base addition, no mortality occurred in neutralized waters. Relatively high Ca concentrations may have mitigated potential toxic effects from Al oversaturation. Plasma Cl levels did not vary with distance downstream. Detoxification of running waters with highly toxic levels of pH and Al can successfully be performed by addition of limestone slurry.

Des tacons du saumon de l'Atlantique (*Salmo salar*) ont été exposés pendant 70 h aux eaux acides de la rivière Vaaraana dans le sud de la Norvège (Q = 1,3 m^3/s, pH = 4,4–5,6, Ca = 0,6–1,5 mg/L, Al labile = 50–100 μg/L), et à des eaux neutralisées par des boues calcaires. Les paramètres chimiques et biologiques ont été mesurés en amont du point de chaulage et à 20, 100, 1 000, 2 000 et 3 500 m en aval. L'ajout des boues a augmenté le pH de 4,6 à 6,9, le Ca de 0,6 à 2,6 mg/L et réduit la concentration de l'Al labile de 59 à 35 μg/L immédiatement en aval du point de chaulage (20 m, après 30 s). Dans les eaux non traitées d'amont, les tacons perdaient leur Cl plasmatique rapidement, et tous les poissons mouraient dans les 36 h. En dépit de la légère sursaturation de l'Al labile immédiatement en aval du point de traitement, aucune mortalité n'est survenue dans les eaux neutralisées. Les concentrations relativement élevées de Ca peuvent avoir pallié les effets potentiellement toxiques d'une sursaturation d'Al. Les concentrations de Cl dans le plasma n'ont pas varié en fonction de la distance en aval. La détoxification des eaux courantes trop acides et trop riches en aluminium est donc réalisable par l'ajout de boues calcaires.

Received October 30, 1985
Accepted June 19, 1986
(J8519)

Reçu le 30 octobre 1985
Accepté le 19 juin 1986

Many fish species are dependent on running waters (brooks and rivers) for recruitment, and recruitment failure has been identified as a primary cause for salmonid declines in acid waters (Jensen and Snekvik 1972; Rosseland et al. 1980). Since acidic waters were recognized as a major problem for salmonid fish in Norway in the 1920's (Huitfeldt-Kaas 1922; Dahl 1927), various neutralization procedures have been developed and tested (Rosseland and Skogheim 1984a). As a result, restoration of a self-reproducing fish population in an acidic lake is likely to require not only neutralization of the lake itself, but also neutralization of tributary brooks and rivers.

In Norway, Torgersen (1934) first presented methods for neutralizing running waters. Waters were diverted from the stream through a ribbed barrel containing pebble-size lime-

[1]Present address: Norsk Biotech A/S, P.O. Box 788, Krossen, N-4301 Sandnes, Norway.

stone. Later, systems using wells filled with limestone or shell-sand were introduced (Kalleberg 1976). Continuous dosing systems, dispensing fine-grained limestone, were in use as early as the 1930's (Sømme 1942). Experiments at the River Audna, with more advanced technology and dosing systems for limestone slurry, were initiated in 1982 (Abrahamsen and Matzow 1984). Dickson (1983), however, suggested that liming of running water could create a toxic zone caused by changes in aluminum species downstream from the point of liming. Experiments have therefore been performed to investigate the effects of this neutralization procedure on the survival and physiology of smolts of Atlantic salmon (*Salmo salar*).

Materials and Methods

Study Area

The River Audna, situated in the county of Vest Agder, southern Norway (Sørlandet), has a catchment area of 460 km^2. The history of the salmon fishery in the river is typical of that for other major rivers in the region: a rapid decrease in salmon catches during the late 1960's and by the early 1970's, no natural reproduction and few adult fish (Leivestad et al. 1976).

Liming experiments were conducted in the River Vaaraana, a tributary of the River Audna. The River Vaaraana, located 40 km from the coast, has a catchment area of 33.5 km^2, of which 18.5 km^2 is situated upstream of the point of liming. The River Vaaraana, with a mean flow of 1.3 m^3/s, is chronically acidic (pH = 4.4−5.6, Ca = 0.6−1.5 mg/L, total Al = 250−350 μg/L, labile Al = 50−100 μg/L).

Pulverized $CaCO_3$ (grain-size 0−20 μm, 50% <2 μm in diameter) held in suspension with dispersal agents (aminformate and polyacrylate, concentration of 0.7%, not known to be harmful to fish) was added to the river. The dosing system, described in Abrahamsen and Matzow (1984), automatically adjusted dosing to water flow.

Six study sites were established: one upstream of the limestone slurry addition and five downstream at 20, 100, 1000, 2000, and 3500 m below the dosing point. During the experiment, water reached station 2 (20 m downstream) about 30 s after the addition of the limestone slurry. Approximately 90 min passed before the water reached station 6 (3500 m downstream).

Experimental Fish

Fish used in the experiment were 1-yr-old smolts of Atlantic salmon of the River Imsa strain, raised at the Research Station for Freshwater Fish at Ims, southwestern Norway (pH = 6.5, Ca = 1.5 mg/L, labile Al = 20 μg/L). A representative sample (n = 97) of fish averaged (±SD) 14.3 (±0.9) cm in length and 27.8 (±9.6) g in weight. Fish were not fed during the experiment.

The day before the start of the experiment, fish were transported in fish tanks by car and held overnight in a large keepnet (volume 2 m^3) 1000 m downstream from the point of liming (station 4). The liming system was operating when fish entered into the river. The following day, salmon smolts were transferred to keepnets (maximum 15 smolts per net, net volume 40 L) at five additional sites in the river. Keepnets were sheltered from heavy stream flow by a wood plow. Adjustable openings in front of the plow allowed for adjustment of water flow. Flow behind the plow, measured with a flowmeter (Shieltknecht, Micro Mini Water 1), was adjusted to 0.1 m/s. Each station contained three keepnets: 10 fish for mortality and 30 fish for blood sampling.

The experiment lasted 70 h. At intervals, blood samples (n = 4) were drawn by heart puncture. Samples were centrifuged immediately (MSE Micro hematrocrit centrifuge type 346, 3 min) and analyzed for hematocrit and plasma Cl (Radiometer CMT 10, chloride titrator). Gills of fish used for blood sampling were visually checked for mucus clogging.

Water Chemistry

Water samples were collected at all locations at 15, 26, 34, 48, and 70 h after the start of the experiment. Samples were immediately analyzed for pH, prepared for Al analyses, and filtered (0.45 μm). pH was measured with an Orion model 207 pH meter with a Radiometer glass electrode. Aluminum was analyzed by the pyrocatechol violet method (Dougan and Wilson 1974), and the fraction obtained without any pretreatment is denoted acid-reactive Al. Since the colour complex, acid strength, and reaction time are different from the hydroquinoline method used by Driscoll (1980), none of the analytical fractions reported here are identical to those of Driscoll. Samples for Al analyses were passed through a cation-exchange column (DOWEX-50, 20/50 mesh, prepared with Na^+) and analyzed for Al. This fraction is called nonlabile Al and is assumed to primarily include organically complexed monomeric Al. In water with a low concentration of organic matter undergoing rapid pH change, nonlabile Al may include newly precipitated inorganic Al forms as well. The difference between the acid-reactive Al and the nonlabile Al is termed labile Al and assumed to include inorganic monomeric species of Al. This fraction is analogous to the labile Al fraction of Driscoll (1980), as it represents Al forms retained in a cation-exchange column. The labile Al fraction is considered to include the toxic Al forms (Driscoll et al. 1980). Total Al was measured as acid-reactive Al after digestion of aliquots of the sample in $K_2S_2O_8$.

Calcium was determined on unfiltered and filtered (0.45 μm) water samples by atomic absorption spectrophotometry (Perkin Elmer model 603). Bicarbonate alkalinity was determined by automatic titration with 0.02 M HCl using a Radiometer RTS 822 and Gran's plot. Sulphate and Cl were analyzed by an ion-exchange technique combined with conductometry (modified after Stainton 1974). Fluoride (total) was analyzed by an ion-selective electrode (Orion 1976). Dissolved organic matter was estimated by UV absorbance (Uvicord LKB 8300) at 254 nm with a 1-cm cuvette.

Calculation of Al Speciation

Estimated concentrations of inorganic monomeric species of Al were calculated, assuming chemical equilibrium, from measurements of pH, F, sulphate, and labile Al. Calculations were performed with the computer program MINEQL (Westall et al. 1976). Ionic strength was estimated to be 4.0−6.7 mM. Equilibrium constants used for the inorganic complexes were similar to those compiled by Driscoll (1980). Previous studies (Wright and Skogheim 1983; Skogheim et al. 1984; Rosseland and Skogheim 1984b) have indicated that the solubility of Al in Norwegian waters is controlled by a form of $Al(OH)_3$, with

TABLE 1. Water chemistry during the experiment in River Vaaraana at stations 1–6. Data represent means ± SD with number of samples given in parentheses.

Station	pH	Total Ca (mg/L)	Dissolved Ca (mg/L)	Total Al (μg/L)	Acid-reactive Al (μg/L)	Nonlabile Al (μg/L)	Labile Al (μg/L)
1	4.64±0.02 (5)	0.58±0.02 (3)	0.58±0.02 (3)	250±17 (4)	172±8 (4)	113±6 (4)	59±8 (4)
2	6.69±0.22 (5)	3.23±1.22 (3)	2.49±0.60 (3)	247±11 (4)	211±6 (4)	176±8 (4)	35±5 (4)
3	6.69±0.23 (5)	2.72±0.63 (3)	2.45±0.36 (3)	253±22 (4)	210±8 (4)	177±4 (4)	33±9 (4)
4	6.55±0.15 (5)	2.59±0.39 (3)	2.55±0.37 (3)	254±16 (4)	215±9 (4)	195±8 (4)	20±6 (4)
5	6.61±0.16 (5)	2.69±0.30 (3)	2.68±0.28 (3)	262±17 (4)	215±12 (4)	194±10 (4)	21±5 (4)
6	6.26±0.17 (5)	1.84±0.1 (3)	1.84±0.10 (3)	234±19 (4)	195±4 (4)	176±8 (4)	19±8 (4)

a solubility constant similar to that measured for synthetic gibbsite ($K_{SO} = 10^{9.33}$ at 4.8°C).

Results and Discussion

Water Quality

Water chemistry upstream of the liming site (station 1) was relatively constant during the experiment, with mean values pH = 4.64, Ca = 0.58 mg/L, total Al = 250 μg/L, acid-reactive Al = 172 μg/L, and labile Al = 59 μg/L (Table 1).

Downstream of the limestone slurry addition, pH increased immediately. The highest pH values, on the average, occurred at station 2 (mean pH = 6.69), with pH decreasing to 6.26 at location 6, 3500 m downstream. Total Ca concentration increased to 3.23 mg/L at station 2, but only 77% (2.49 mg/L) of this Ca was dissolved. All particulate Ca had dissolved by station 4 (1000 m downstream) (Table 1; Fig. 1).

Bicarbonate alkalinity levels in the River Vaaraana were 0 meq/L. After liming, alkalinity increased to levels between 0.025 and 0.100 meq/L. The regression between alkalinity (Alk) in filtered water at stations 2–5 and the increase in dissolved Ca relative to station 1 (△ dissolved Ca) was

$$\text{Alk} = 0.967 \cdot \triangle \text{ dissolved Ca} - 0.033$$

with $r = 0.973$ and $n = 20$. Both variables are given in milliequivalents per litre. The correlation coefficient is highly significant ($p < 0.001$). The slope of the regression is near the theoretical value of 1.0. To obtain pH = 5.50 with no bicarbonate alkalinity (= 0.000), 0.033 meq/L of bases is needed. Neutralization of strong acids accounts for 0.023 meq/L; Al and dissolved organic matter accounts for the remaining 0.010 meq/L.

Total Al was relatively constant over time and similar at all locations, but there was a relative change in Al fractions. The acid-reactive and nonlabile Al increased after slurry addition, whereas the labile Al decreased (Table 1; Fig. 1).

Total F was measured only at station 1 (mean value = 45 μg F/L). At station 1 (pH = 4.64), all F was complexed by Al. At stations 2–6 (pH = 6.69–6.26), on the other hand, an insignificant fraction of the F was complexed by Al.

Theoretical concentrations of labile Al in equilibrium with a solid phase with solubility similar to synthetic gibbsite are compared with labile Al levels measured in the River Vaaraana, above and below limestone slurry additions (Fig. 2). Calculations of theoretical equilibrium Al concentrations assume that the solubility of the aquo aluminum ion (Al^{3+}) is controlled by a solid phase aluminum trihydroxide ($Al(OH)_3^0$), with the solubility constant $K_{s0} = 10^{9.33}$ at the measured experimental temperature of 4.8°C. Relative to these calculated equilibrium concentrations, measured labile Al levels indicated a high degree of undersaturation at station 1 (upstream), a slight oversaturation at stations 2 and 3 (immediately downstream), and near equilibrium at stations 4, 5, and 6 (Fig. 2). The dominant form of Al in the neutralized water was $Al(OH)_4^-$ (aluminate ion).

Liming did not influence the levels of sulphate and Cl. Concentrations of both components were constant at 2.5 mg/L throughout the experiment. Dissolved organic matter, estimated as absorbance of UV light, was relatively constant at all stations throughout the experiment. Values varied between 0.108 and 0.128 cm^{-1}.

Fish Response

Levels of plasma Cl in salmon at the start of the experiment were lower (110 ± 5 meq Cl/L) than in smolts of the same population under normal conditions (120–130 meq Cl/L) (Rosseland and Skogheim 1982, 1984b), perhaps in part due to stress caused by transportation (Soivio and Virtanen 1984). Handling and confinement have been shown to induce loss of plasma Cl in coho salmon (*Oncorhynchus kisutch*) and steelhead trout (*Salmo gairdneri*) (Wedemeyer 1972; Redding and Schreck 1983), and largemouth bass (*Micropterus salmoides*) (Carmichael et al. 1984).

In the natural river water (station 1), all salmon smolts died within 36 h (L_{50} = 22 h). No mortality occurred at any sites downstream of the limestone slurry additions (Fig. 3). Our results agree with those of Abrahamsen and Matzow (1984), testing fingerlings of Atlantic salmon and sea-running brown trout (*Salmo trutta*).

At station 1, fish lost plasma Cl very rapidly (△Cl = 1.9 meq/h) (Fig. 3), reflecting the toxicity of the unlimed river water. During the experiment, pH levels ranged from 4.6 to 4.7 and levels of labile Al from 50 to 65 μ,g/L. The rapid mortality of salmon smolts at these relatively low levels of labile Al was somewhat unexpected. Previous experiments have indicated that concentrations of labile Al between 313 and 462 μg/L result in similar rapid decreases in plasma Cl (Rosseland and Skogheim 1982, 1984b). Although pH levels in this experi-

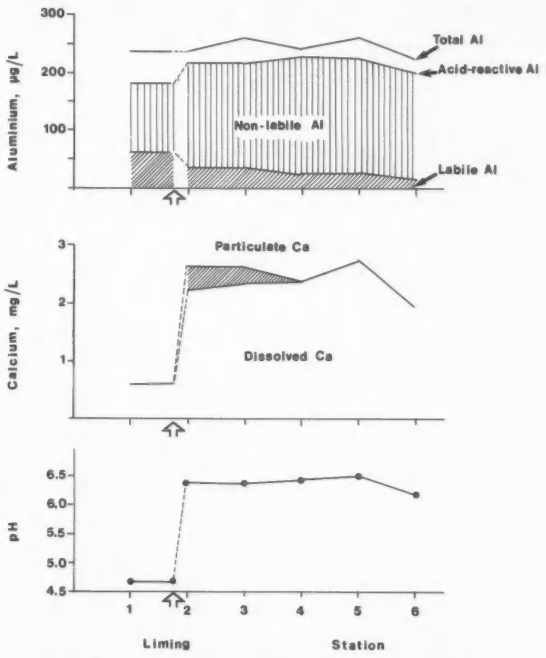


FIG. 1. Concentrations of Al fractions, Ca, and pH from stations 1–6 70 h after the start of the experiments.

ment (pH = 4.6) were lower than in previous experiments (pH = 4.9), we believe that low Ca concentrations in the River Vaaraana are the primary reason for the extreme toxicity of the untreated water in this experiment relative to mortalities observed in earlier experiments. Calcium levels in the River Vaaraana averaged 0.6 mg/L, as compared with 1.7 mg/L in experiments reported by Rosseland and Skogheim (1982, 1984b). The sensitivity of fish to acidic waters with low Ca concentrations was demonstrated by the fishkill observed in the River Vikedal, southwestern Norway; salmon presmolts died during an episode with pH = 5.1–5.3, Ca = 0.5–0.9 mg/L, and labile Al = 30–40 µg/L (Henriksen et al. 1984). Leivestad et al. (1980) and Brown (1983) experimentally confirmed that Ca mitigates the toxicity of low pH and high Al concentrations to brown trout, although the beneficial effects of Ca appeared "saturated" at Ca concentrations of 0.9–1.7 mg/L (Leivestad et al. 1980) and 0.5–1.0 mg/L (Brown 1983).

In laboratory experiments, Baker and Schofield (1982) observed that oversaturated Al solutions were particularly toxic to fish. Dickson (1983) observed increased fish mortalities in lakes shortly after liming and proposed that this mortality resulted from toxicity of waters oversaturated with Al.

In contrast, in our experiment, no mortality occurred at any location downstream of the treatment site. At stations 2, 3, 5, and 6, an initial drop in plasma Cl occurred within the first 24 h of the experiment, from a mean of 110 ± 5 meq/L to between 85 and 100 meq/L.

The transient depletion of plasma Cl observed during the first 24 h of the experiment at all downstream locations likely resulted from further handling and confinement stress associated with transfer to the small keepnets at stations 2, 3, 5, and 6.

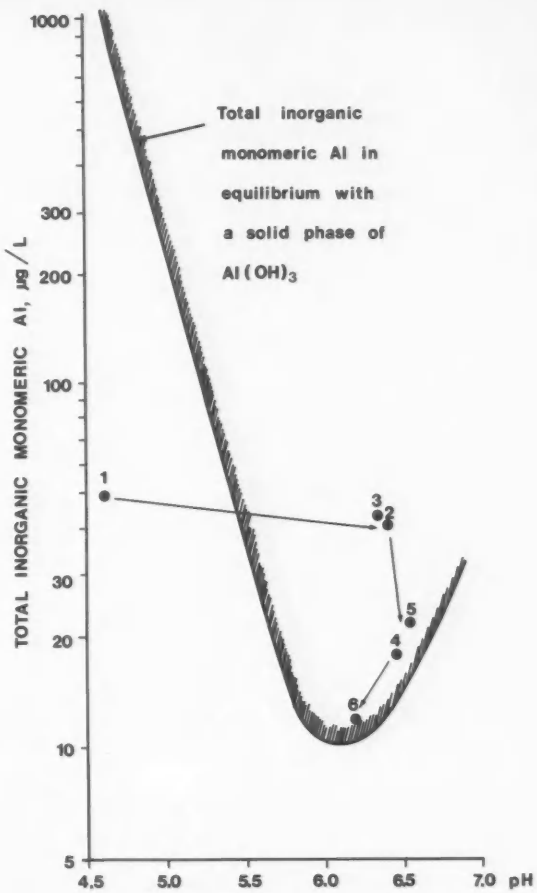


FIG. 2. Concentrations of labile Al as a function of pH at stations 1–6. Theoretical equilibrium concentrations of inorganic monomeric Al species are calculated using the solubility constant of synthetic gibbsite applicable at 4.8°C ($K_{SO} = 10^{9.33}$) and equilibrium constants from Driscoll (1980). Arrows indicate the time sequence.

Although fish in the large keepnet at station 4 were tested less frequently than fish at other sites, a significantly lower level of plasma Cl (Student t-test, $p < 0.01$) was also observed in these fish at 25 h, reflecting stress due to removal of fish with landing nets for transfer to the other locations. Except for station 5, plasma Cl levels at 25 h were significantly higher in fish in the large keepnet (station 4) than in fish in small keepnets at the other downstream locations. Stress caused by confinement appeared to be lower in the large keepnet. During the remainder of the experiment, plasma Cl levels were not significantly different among stations 2–6. These comparisons indicate that the transient ion depletion observed in neutralized water resulted solely from handling and confinement stress.

In untreated river water (station 1), plasma Cl losses may in part be due to handling and confinement stress. The magnitude of the ion loss rate at station 1, relative to stations 2–6, however, suggests that these stress factors are of minor importance as compared with stress associated with pH and Al toxicity.

The mean value (±SD) for hematocrit at the start of the experiment was 34 (±4). Hematocrit levels increased signifi-

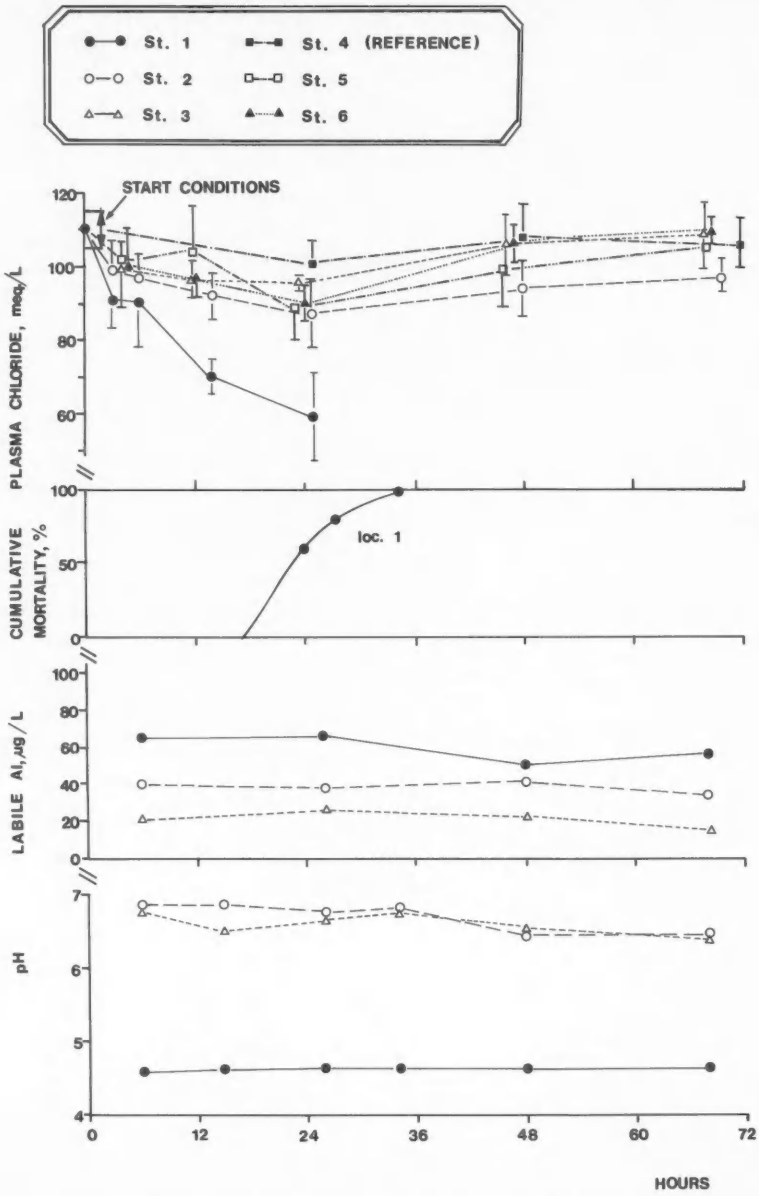


FIG. 3. Physiological stress, measured as changes in plasma Cl, and mortality of Atlantic salmon smolts above (station 1) and below (station 2–6) additions of limestone slurry to the River Vaaraana. The mean values (±SD) for plasma Cl within each sample are indicated ($n = 4$, except at station 4 where $n = 5$). For other parameters, see Table 1.

cantly (Student t-test, $p < 0.001$) in proportion to the decrease in plasma Cl (Fig. 4), a phenomenon also observed by others (e.g. Neville 1979; McDonald et al. 1980; Giles et al. 1984; Rosseland and Skogheim 1984b). According to Wood and McDonald (1982), the increased hematocrit may result from a combination of three factors: reduced plasma volume, red blood cell (RBC) swelling, and RBC mobilization. The relative importance of these three factors was not evaluated in this experiment. The computed regression line was not significantly different ($p > 0.1$) from that obtained by Rosseland and Skogheim (1984b) with data on Atlantic salmon smolts of the same strain, and hence seems representative for this strain of salmon exposed to various levels of pH and Al.

As in other experiments with acidified waters (Rosseland and Skogheim 1984b) and during episodic fish kills in nature (Skogheim et al. 1984), gills from newly dead and dying fish

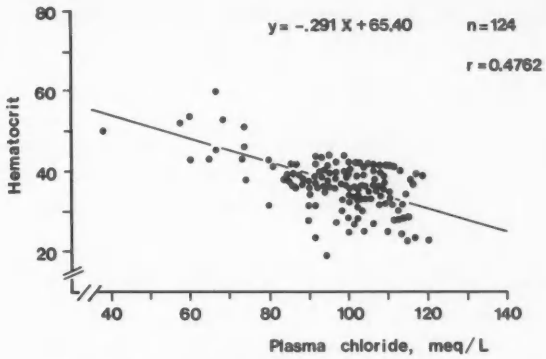


FIG. 4. Relationship between values of hematocrit (HCT) and plasma Cl at stations 1–6 ($n = 124$). The regression line (HCT = −0.291 Cl + 65.40, $r = 0.476$, $p < 0.001$) is given.

and fish taken for blood sampling showed no signs of excess mucus at any location.

We conclude that running waters with highly toxic levels of pH and Al can successfully be neutralized and detoxified by means of limestone slurry.

Acknowledgements

We gratefully acknowledge the assistance given in field by Kjell Jakob Andersen and the valuable comments on the manuscript given by the Drs. Joan P. Baker, Arne Henriksen, and Richard F. Wright. The study was partly financed by the Norwegian Liming Project.

References

ABRAHAMSEN, H., AND D. MATZOW. 1984. Use of lime slurry for de-acidification of running water. Verh. Int. Ver. Limnol. 22: 1981–1985.

BAKER, J. P., AND C. L. SCHOFIELD. 1982. Aluminum toxicity to fish in acidic waters. Water Air Soil Pollut. 19: 289–309.

BROWN, D. J. A. 1983. Effects of calcium and aluminium concentration on the survival of brown trout (*Salmo trutta*) at low pH. Bull. Environ. Contam. Toxicol. 30: 382–387.

CARMICHAEL, G. J., J. R. TOMASSO, B. A. SIMCO, AND K. B. DAVIS. 1984. Confinement and water quality-induced stress in largemouth bass. Trans. Am. Fish. Soc. 113: 767–777.

DAHL, K. 1927. The effects of acid water on trout fry. Salmon Trout Mag. 46: 35–43.

DICKSON, W. 1983. Liming toxicity of aluminium to fish. Vatten 39: 400–404.

DOUGAN, W. K., AND A. L. WILSON. 1974. The absorptiometric determination of aluminium in water. A comparison of some chromogenic reagents and the development of an improved method. Analyst 99: 413–430.

DRISCOLL, C. T. 1980. Chemical characterization of some dilute acidified lakes and streams in the Adirondack Region of New York State. Ph.D. thesis, Cornwell University, Ithaca, NY.

DRISCOLL, C. T., J. P. BAKER, J. J. BISOGNI JR., AND C. L. SCHOFIELD. 1980. Effects of aluminium speciation on fish in dilute acidified waters. Nature (Lond.) 284: 161–164.

GILES, M. A., H. S. MAJEWSKI, AND B. HOBDEN. 1984. Osmoregulatory and hematological responses of rainbow trout (*Salmo gairdneri*) to extended environmental acidification. Can. J. Fish Aquat. Sci. 41: 1686–1694.

HENRIKSEN, A., O. K. SKOGHEIM, AND B. O. ROSSELAND. 1984. Episodic changes in pH and aluminium-speciation kill fish in a Norwegian salmon river. Vatten 40: 255–260.

HUITFELDT-KAAS, H. 1922. Om aarsaken til massedød av laks og ørret i Frafjordelven, Helleelven og Dirdalselven i Ryfylke høsten 1920 (On the cause of mass death of salmon and trout in Frafjordelven, Helleelven and Dirdalselven in Ryfylke, autumn 1920). Norsk Jæger- og Fiskefor., tidsskrift, p. 37–44. (In Norwegian)

JENSEN, K. W., AND E. SNEKVIK. 1972. Low pH levels wipe out salmon and trout populations in southern Norway. Ambio 1: 223–225.

KALLEBERG, H. 1976. Praktiske erfaringer ved kalking av fiskevann (Experiences from liming of lakes). Vann 1: 62–69. (In Norwegian)

LEIVESTAD, H., G. HENDREY, I. P. MUNIZ, AND E. SNEKVIK. 1976. Effects of acid precipitation on freshwater organisms, p. 87–111. *In* F. H. Brække [ed.] Impact of acid precipitation on forest and freshwater ecosystems in Norway. SNSF-project, FR 6/76.

LEIVESTAD, H., I. P. MUNIZ, AND B. O. ROSSELAND. 1980. Acid stress in trout from a dilute mountain stream, p. 318–319. *In* D. Drabløs and A. Tollan [ed.] Ecological impact of acid precipitation. Proc. Int. Conf., Sandefjord, Norway, 1980.

MCDONALD, D. G., H. HOBE, AND C. M. WOOD. 1980. The influence of calcium on the physiological responses of the rainbow trout, *Salmo gairdneri*, to low environmental pH. J. Exp. Biol. 88: 109–131.

NEVILLE, C. M. 1979. Sublethal effects of environmental acidification on rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 36: 84–87.

ORION. 1976. Instruction manual. Flouride electrodes. Orion Research Inc., Cambridge.

REDDING, J. M., AND C. B. SCHRECK. 1983. Influence of ambient salinity on osmoregulation and cortisol concentration in yearling coho salmon during stress. Trans. Am. Fish. Soc. 112: 800–807.

ROSSELAND, B. O., I. SEVALDRUD, D. SVALASTOG, AND I. P. MUNIZ. 1980. Studies of freshwater fish populations — effects of acidification on reproduction, population structure, growth and food selection, p. 336–337. *In* D. Drabløs and A. Tollan [ed.] Ecological impact of acid precipitation. Proc. Int. Conf., Sandefjord, Norway, 1980.

ROSSELAND, B. O., AND O. K. SKOGHEIM. 1982. Physiological stress and mortality of Atlantic salmon, *Salmo salar* L. in acid water with high levels of aluminium. ICES, C.M. 1982/M:29: 15 p.

1984a. Attempts to reduce effects of acidification on fishes in Norway by different mitigation techniques. Fisheries 9: 10–16.

1984b. A comparative study on salmonid fish species in acid aluminium-rich water. II. Physiological stress and mortality of one and two year old fish. Rep. Inst. Freshwater Res. Drottningholm 61: 186–194.

SKOGHEIM, O. K., B. O. ROSSELAND, AND I. SEVALDRUD. 1984. Deaths of spawners of Atlantic salmon in River Ogna, SW Norway, caused by acidified aluminium-rich water. Rep. Inst. Freshwater Res. Drottningholm 61: 195–202.

SOIVIO, A., AND E. VIRTANEN. 1984. Physiological effects of stocking stress on Baltic salmon (*Salmo salar*). EIFAC Tech. Pap. No. 42, Suppl., Vol. 1: 217–223.

STAINTON, M. P. 1974. An automated method for determination of chloride and sulphate in freshwater using cation exchanges and measurement of electric conductance. Limnol. Oceanogr. 19: 707–711.

SØMME, I. D. 1942. Ørretboka (The trout). Jacob Dybwads forlag, Oslo. 591 p.

TORGERSEN, H. 1934. Forsøk med opparbeidelse av sure, fisketomme vann (Attempts to restock acid, barren lakes). Stangfiskeren: 38–46. (In Norwegian)

WEDEMEYER, G. 1972. Some physiological consequences of handling stress in the juvenile coho salmon (*Oncorhynchus kisutch*) and steelhead trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 29: 1780–1783.

WESTALL, J. C., J. L. ZACJARY, AND F. N. M. MOREL. 1976. MINEQL, a computer program for the calculation of chemical equilibrium composition of aqueous systems. Dep. Civ. Eng. MIT Tech. Note No. 18.

WOOD, C. M., AND D. G. MCDONALD. 1982. Physiological mechanisms of acid toxicity to fish, p. 197–226. *In* R. Johnson [ed.] Proc. Int. Symp. Acidic Rain and Fishery Impact on Northeastern North America. American Fisheries Society, Bethesda, MD.

WRIGHT, R. F., AND O. K. SKOGHEIM. 1983. Aluminium speciation at the interface of an acid stream and a limed lake. Vatten 39: 301–304.

A New View of the Predation Cycle of a Planktivorous Fish, White Crappie (*Pomoxis annularis*)

W. John O'Brien, Barbara I. Evans, and Gregory L. Howick

Department of Systematics and Ecology, University of Kansas, Lawrence, KS 66045, USA

O'Brien, W. J., B. I. Evans, and G. L. Howick. 1986. A new view of the predation cycle of a plantivorous fish, white crappie (*Pomoxis annularis*). Can. J. Fish. Aquat. Sci. 43: 1894–1899.

Predators are often categorized as either cruise or ambush feeding strategists. We present evidence that white crappie (*Pomoxis annularis*) are neither. Instead, the crappie swim intermittently and search only when stationary. If the crappie searched while swimming, one would expect the run speeds to be slower than the pursuit speeds, but no difference was found between these two measurements. Assuming that prey are located while swimming, a foreshortening of runs prior to pursuit would also be expected, but again, none was detectable. The duration of the search pause appears to be related to the detectability of the prey. Crappie also search during the pause immediately following the attack and ingestion of a prey item. The observation that the probabilities of detecting and pursuing a prey following a run or an attack do not differ significantly supports this conclusion. Also, the duration of the pause following a run or attack does not differ significantly over a wide range of temperatures. If these views are correct, white crappie could not forage optimally by either deleting located prey items from the diet or minimizing handling time. What they appear to be doing is creatively managing their search time.

On considère souvent que les prédateurs utilisent pour se nourrir une stratégie de croisière ou d'embuscade. Nous présentons des données montrant que la marigane blanche (*Pomoxis annularis*) n'entre dans aucune de ces catégories. Ce poisson nage de façon intermittente et ne cherche sa nourriture que lorsqu'il est stationnaire. Si la marigane chassait en nageant, on s'attendrait à ce que la vitesse de croisière soit inférieure à la vitesse de poursuite, mais aucune différence n'a été notée entre ces deux mesures. Si les proies étaient localisées pendant la nage, on pourrait aussi prévoir un arrêt du mouvement avant la poursuite mais, là encore, cela n'a pu être observé. La durée de la pause de recherche semble liée à la détectabilité de la proie. La marigane cherche aussi pendant la pause qui suit immédiatement l'attaque et l'ingestion d'une proie. Nous avons observé que les probabilités de détection et de poursuite d'une proie tout de suite après un déplacement ou une attaque ne diffère pas de façon significative, ce qui étaye notre conclusion. D'autre part, la durée de la pause qui suit un déplacement ou une attaque ne diffère pas de façon significative à des températures diverses. Si notre opinion est juste, ce n'est pas en éliminant de leur régime alimentaire certaines proies localisées ni en minimisant le temps de « manutention » que la marigane optimalise sa recherche de nourriture. Ce qu'elle semble faire consiste à gérer créativement son temps de recherche.

Received November 8, 1985
Accepted June 23, 1986
(J8559)

Reçu le 8 novembre 1985
Accepté le 23 juin 1986

The feeding of predators is one of the most important aspects of ecology and is often an action that profoundly affects the surrounding community (Paine 1969; Curio 1976). Two of the most important aspects of predation are what is being eaten and the rate of consumption. We have analysed the factors controlling the diet composition of planktivorous white crappie (Wright and O'Brien 1982, 1984; in prep.). Here we analyse factors controlling the rate of consumption.

Predators are often categorized as ambush or cruise predators, according to their feeding strategy. *Chaoborus* larvae are freshwater invertebrate ambush planktivores (Pastorok 1980). Plantivorous fish have been thought to be cruise predators, continuously searching as they swim (Confer and Blades 1975). However, planktivorous feeding white crappie (*Pomoxis annularis*) do not swim continuously but alternate short swimming bursts and pauses. Here we present evidence that they search only when stationary and, therefore, cannot be classified as either ambush or cruise predators. This has important ramifications when estimating feeding rate and when considering how the fish may have evolved to forage optimally.

Methods

Search behavior of white crappie was examined by observing four fish (9–15 cm TL), feeding two at any given time, in a 90 × 90 × 90 cm aquarium filled to a depth of 30 cm. The outside of the aquarium was covered with white styrofoam to insulate it and to visually isolate the fish from external distractions. The behavior of the fish was videotaped using a JVC 100 color camera mounted 1.5 m above the bottom of the tank. The field of view on the television screen encompassed 90% of the aquarium's bottom. As the fish fed, each action was described and recorded onto the audio portion of the tape by two observers watching through small windows located on opposite sides of the aquarium.

Prior to an experiment, the fish were starved for at least 24 h. Experiments consisted of adding to the aquarium a known number of either small lake zooplankton or large cultured zooplankton (*Daphnia magna* or *Daphnia pulex*). The lake zooplankton prey assemblage ranged in size from 0.5 to 1.5 mm and was composed mainly of *Daphnia galeata*, *Daphnia ambigua* and *Diaptomus* spp. (primarily *Diaptomus pallidus*).

TABLE 1. Percentage of times a stop or pause is followed by a run or a pursuit as visual densities change (all observations at 19–20°C and light > 100 lx).

Row No.	Prey type	Density (no./L)	Visual density	% stops			
				Followed by pursuit	95% C.I. (LL→UL)	Followed by run	95% C.I. (LL→UL)
1	Small prey (lake daphnids, $n = 273$)	70	43.5	84.6	80.0→89.2	15.4	10.8→20.1
2	Large prey (*D. magna*, $n = 67$)	1.0	42.4	70.2	57.6→80.7	29.8	19.3→42.6
3	Large prey (*D. magna*, $n = 83$)	0.1	4.2	65.1	53.5→73.3	34.9	24.7→46.5
4	Small prey (lake daphnids, $n = 62$)	5	3.1	37.1	24.9→50.4	62.9	49.6→75.1
5	Small prey (lake copepods, $n = 68$)	5	3.1	25.0	15.1→37.3	75.0	62.7→84.9

When using lake zooplankton, experimental densities were approximately 5 zooplankters/L^{-1}. When *D. magna* were used, the densities in the aquarium were either 0.1 or 1.0 prey/L and organisms ranged in size from 3 to 4 mm. In experiments run at a density of 0.1 prey/L, a new prey was introduced into the aquarium by siphon each time the fish ate a daphnid, thereby avoiding prey depletion. When *D. pulex* were used, the density was 7.0 prey/L and the *D. pulex* ranged in size from 2.0 to 3.0 mm.

The light in the aquarium came from two rheostat-controlled 150-W incandescent flood lights and was diffused by a thick white cloth arranged tentlike above the aquarium with the TV camera projecting through. Light intensity was maintained between 100 and 300 lx, at or above the maximum reaction distance threshold of 100 lx observed for white crappie (Wright and O'Brien 1984). The temperature in the aquarium was held constant by using a large aquarium heater and/or Blue M cold finger, both regulated by a Technilab thermostat. Fish were observed at temperatures ranging from 7 to 24°C and were acclimated to a new temperature for at least 1 d/1°C temperature change. All observations were made during the early afternoon.

White crappie swim intermittently while feeding, swimming short distances and then stopping for a short period of time. Swimming followed by an attack on prey is termed a "pursuit," but if no attack follows, this swimming behavior is termed a "run." The stationary pauses between swimming movements are believed to be used for search. The videotapes were analysed by assigning the actions of the fish to one of the components of the feeding cycle: search, run, pursuit, or attack. The duration of each component was timed from the videotapes using a Cronus digital stopwatch. Distances were measured on the TV screen and converted into real units by multiplying by the ratio of actual fish length to TV fish length. Run and pursuit speeds were then reported in centimetres per second. Graphed data points represent means of at least 20 observations and the line indicates the 95% confidence interval. The confidence intervals for the percentage data in Tables 1 and 2 were obtained from statistical tables for binomial samples (Mainland et al. 1956).

When comparing feeding behavior on different prey types, visual densities were used (Table 1). Visual density is calculated by multiplying the volume of the visual field by the absolute density. The visual field for crappie feeding on small lake zooplankton has been found to be 60° (O'Brien and Wright 1985) and 180° when feeding on large daphnids (Evans 1986). For small lake zooplankton the visual field volume was calculated as the volume of a cone with a 60° apex and sides equal to the reaction distance for that prey type (Wright and O'Brien 1984). For *D. magna*, the visual field volume was calculated as the volume of a half-pie with a height of approximately 30 cm. This estimate is based on the observation that crappie rarely pursue prey more than 20° above or below their horizontal plane (Evans and O'Brien 1986). To determine the likely prey handling abilities of crappie, the mouth gape of five 8 cm TL fish was measured. Field-collected, formalin-preserved fish were used. The gape was measured with a ruler by manually expanding the mouth opening until modest resistance was felt.

Results

The feeding behavior of white crappie is shown diagrammatically in Fig. 1. This type of feeding is typical of all planktivorous feeding white crappie. White crappie always pause before pursuing prey. We hypothesize that white crappie do not search while swimming (i.e. neither during a run nor a pursuit) but only search during a pause (i.e. a stationary search). It is also possible that prey are detected during the run rather than during the pause. The pause might simply represent preparation time for the ensuing pursuit. If this were the case, then all pauses should be followed by a pursuit. However, as prey visual densities decrease, the percent of pauses with no pursuit increases (Table 1). If few or no prey are visible, the fish display Sequence I (search–run, search–run) as shown in rows 4 and 5 in Table 1. On the other hand, if prey are likely to be located, the fish primarily display Sequence II (search–pursuit–attack, search–pursuit–attack) as shown in rows 1, 2, and 3 in Table 1.

If the fish search during a run, some of the run lengths prior to a pursuit should be quite short because the fish would occa-

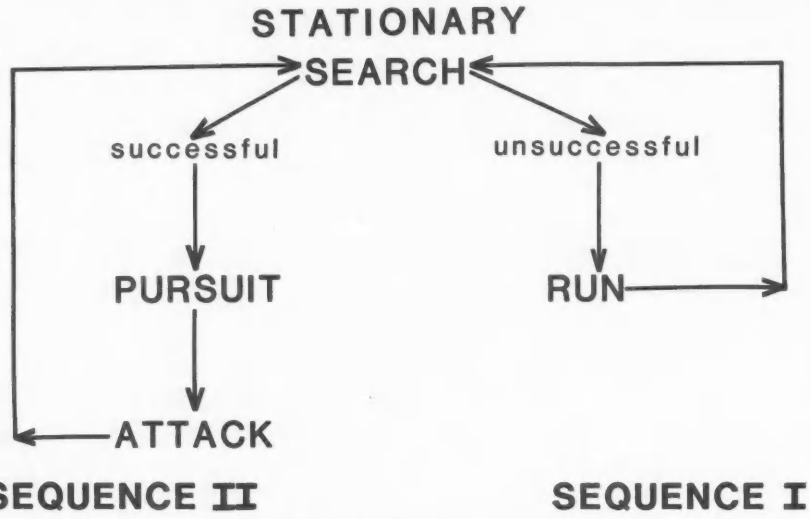


FIG. 1. Current view of the predation cycle of white crappie. Sequence I depicts the series of behaviors which result when the fish search but do not locate a prey. Sequence II depicts the series of behaviors which result when the fish search and do locate a prey.

TABLE 2. Percentage of pursuits following a pause after a run and after a pursuit at five different temperatures. The confidence intervals for the one percentage always encompass the value of the other percentage. Thus, the percentages cannot be shown to be different.

Temperature (°C)	% pursuits after run	95% C.I. (LL→UL)	% pursuits after pursuit	95% C.I. (LL→UL)
24	24.2 (n = 33)	11.1→42.3	27.3 (n = 11)	6.02→61.0
19	38.7 (n = 31)	21.87→51.8	36.8 (n = 19)	16.3→61.6
16	63.6 (n = 33)	45.1→79.6	47.1 (n = 34)	29.8→64.8
11	33.3 (n = 12)	9.9→65.2	22.2 (n = 9)	2.8→60.0
7	34.5 (n = 29)	17.9→54.3	28.6 (n = 14)	8.4→58.1

sionally locate a prey soon after commencing a run. In cases where a prey was not located, the runs should generally be longer (Fig. 2). Figure 2 illustrates the lengths of runs preceding a successful search and the lengths of runs preceding an unsuccessful search. Runs are not foreshortened when followed by a successful search. The means of these two run distributions do not differ significantly (t = 0.23, df = 38, $p > 0.5$), nor do their variances (F_{max} = 1.13, df = 2, 19, $p > 0.1$). Also, the overall frequency distributions of run lengths from these two situations do not differ significantly (Kolmogorov–Smirnov two-sample test, D = 100, df = 20, 20, $p > 0.1$). Thus, there is no indication that run lengths are shortened when followed by a pursuit.

Furthermore, if white crappie search during a run, one might expect that the swimming speed during a run would be slow so as to improve search efficiency. Swimming speed during a run does not differ significantly from pursuit swimming speed for water temperatures ranging from 3 to 24°C (slope: t = 1.65, df = 520, $p > 0.05$; intercept: t = 0.30, df = 520, $p > 0.5$) (Fig. 3).

We believe search occurs both during the pause that follows a run sequence (Sequence I) and the pause that follows a pursuit and attack sequence (Sequence II). If search occurs during the pause immediately after an attack, then we should occasionally observe pursuits directly after this pause. We commonly observed this. Furthermore, if the search following pursuit is equivalent to the search after a run, then the percentage of successful searches in each case should be similar. That is, in a given feeding bout, there should be a similar percentage of successful searches through Sequence I and Sequence II. Over a temperature range from 7 to 24°C, the percentage of pursuits following Sequence I does not differ significantly from the percentage of pursuits following Sequence II (Table 2).

Additionally, the duration of the pause after an attack does not differ significantly from the duration of stationary search following a run (slope: t = 1.01, df = 448, $p > 0.2$; intercept: t = 1.33, df = 448, $p > 0.2$) (Fig. 4). However, the duration of the pause was shorter when the fish were feeding on larger

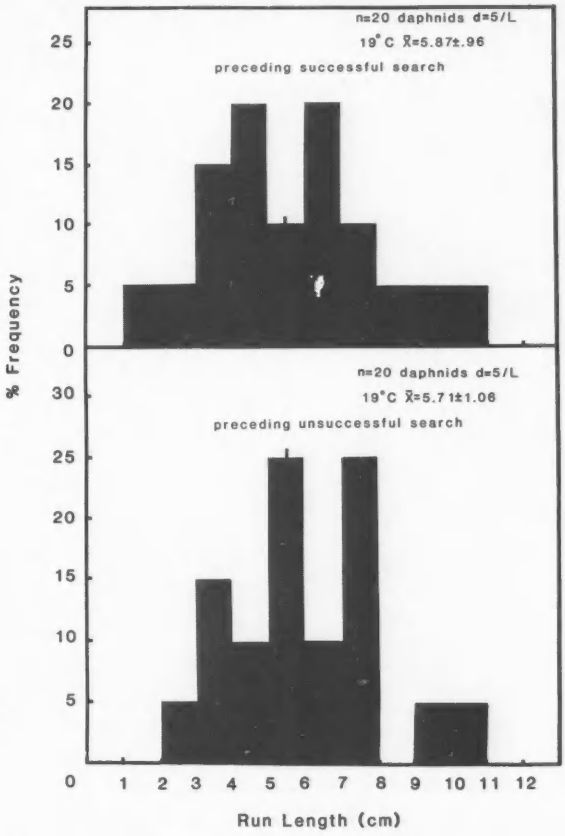


FIG. 2. Frequency of run lengths which were followed by a pursuit (top panel) and frequency of run lengths which were followed by another run (bottom panel).

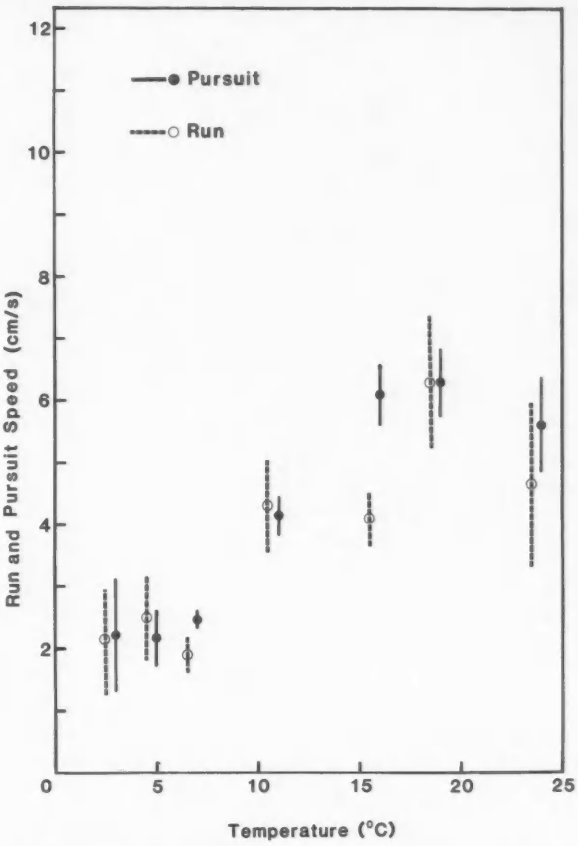


FIG. 3. Run and pursuit speeds measured at temperatures from 3 to 24°C. Points are mean values; lines represent 95% C.I.

prey, *D. magna* (following run: $t = 2.44$, df = 88, $p < 0.02$; following pursuit: $t = 3.43$, df = 89, $p < 0.001$) (Fig. 4).

The mouth size to prey size ratio (MS/PS) for lake zooplankton or *D. magna* is not very large. The mouth gape for an 8 cm TL white crappie was 1 cm. The MS/PS ratio when feeding on lake zooplankton (0.1 cm) is 0.1. When feeding on 0.3-cm *D. magna*, the MS/PS ratio is 0.3.

Discussion

Many investigators interested in the feeding of predators have thought of search as occurring continuously while the predator moves through the environment (Holling 1966; Ware 1975). In this scheme, the volume searched by a planktivorous fish would be a function of the lateral distance at which a prey could be located and the length of the search path (Rosenthal and Hempel 1970; Confer and Blades 1975). This may be thought of as "sweep" search and was first put forth explicitly by Laing (1938). White crappie do not appear to search in this manner but exhibit a move and pause behavior such that search only occurs when the fish is stationary. Janssen (1982) asserted that bluegill sunfish (*Lepomis macrochirus*) search in an identical manner. Some bird species search in this same move-and-search sequence (Smith and Dawkins 1971; Smith 1971; H. R. Pulliam, pers. comm.). The blackbirds studied by Smith (1971) generally moved straight ahead and then stopped, searching by turning their heads. With the exception of head turning movements, white crappie behave similarly. It appears that move-and-search may be quite common and important to an understanding of feeding ecology in several groups of animals.

Not all fish species search in this manner. Some do seem to sweep search (Evans 1986; B. I. Evans and W. J. O'Brien, in prep.). Doubtless, this is true of other animal groups as well. The trade-offs between these two search modes are not obvious. Continuous searching allows the greatest area or volume to be searched if lateral search distance and search efficiency are the same as when stationary. It could be that the run and search mode is adaptive when searching for relatively dense but difficult-to-locate prey, while sweep search is adaptive when searching for relatively sparse but easy-to-locate prey.

The run-and-search mode exhibited by white crappie may be more efficient than it appears. Average reaction distances of crappie feeding on small lake zooplankton are approximately 9 cm (Wright and O'Brien 1984). The average run lengths reported in this paper are about half these reaction distances. Not much water is left unsearched by white crappie, since the run lengths average about half the reaction distance for a given prey type (Evans and O'Brien 1986). Thus, if continuous

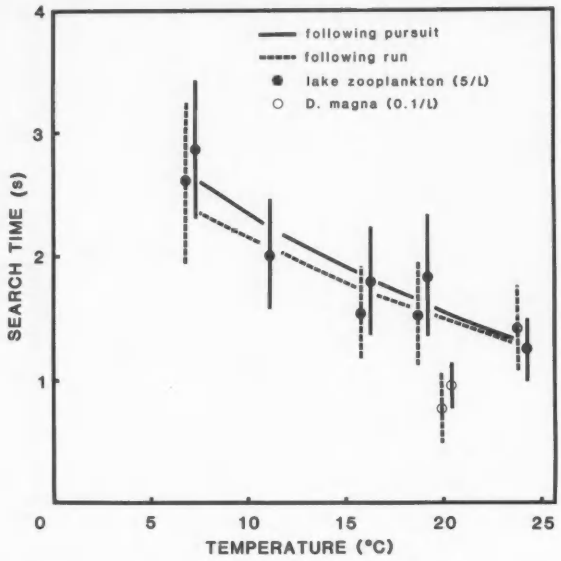


FIG. 4. Stationary search times following a pursuit or a run at temperatures ranging from 7 to 24°C. Points are mean values; lines represent 95% C.I.

search erodes either reaction distance or search efficiency (compared with that of stationary search), then the run-and-search mode may well be the more efficient strategy.

This view of the searching behavior of white crappie has important implications for the use of reaction distance measurements in modeling the feeding of fish which use a run-and-search strategy. In several models of planktivorous fish feeding, the mean reaction distance of the fish to various prey types has been used as the key variable describing their search abilities (Werner and Hall 1974; Confer and Blades 1975; Wright and O'Brien 1984). However, mean reaction distance is actually an underestimate of the maximum distance at which the fish can locate a given prey. This is so because fish are not searching during a run and would commonly have swum past the maximum location distance before pausing to search. As a result, only the maximum reaction distances give an accurate estimate of maximum location distance. Maximum reaction distance would occur in those cases where the fish ended a run just inside the maximum location distance.

Another important aspect of this reappraisal of the predation cycle of white crappie is the contention that the pause following an attack and ingestion of a prey is devoted to search rather than simply handling of the ingested prey. Not only is the time spent in the pause following an attack almost identical to the time spent in stationary search following a run, but the pause is shorter when the fish are feeding on larger prey (Fig. 4). This is contrary to the expectation that the pause would be longer if it were devoted to handling larger prey.

Werner (1974) showed that there was a strong exponential increase in handling time as the MS/PS ratio increased. However, he pointed out that the time was nearly constant at the smaller ratios (0.2−0.5), around 1 s for bluegill sunfish. The MS/PS values for white crappie feeding in these experiments are within the range (0.1−0.3) in which Werner (1974) showed that handling time was constant with increasing prey size. Because the pause following an attack is shorter for large prey than for small prey, there appears to be no time cost for handling zooplankton prey, since handling is occurring during a period of search. This is important if efficient handling time is to be thought of as a means of optimal foraging (Werner and Hall 1974) in fish with this run-and-search feeding strategy.

Several studies have suggested that planktivorous fish may optimize their foraging by deleting items from the diet (Werner and Hall 1974; Eggars 1982). In their view, fish fail to pursue located prey because the cost of the pursuit, attack, and handling is not worth the energy return given that other, more profitable prey are available. In our view, search time will have already been expended and the fish would have to expend energy to reposition through run, should it fail to pursue the prey. It would seem to be energetically advantageous for planktivorous fish to always pursue and attack located prey. Because search follows attack with no time cost in handling, pursuit may be viewed as simply a run with a reward. Instead of minimizing handling time, or deleting certain prey from the diet, white crappie appear to creatively manage the repositioning run length and search time.

Acknowledgements

This work was supported by NSF grant DEB-80-21740. We thank K. Loving, B. Loveless, and L. Johnson for help in carefully and patiently assisting in scoring TV tapes. We thank Shirley Archinal for secretarial assistance and M. O'Brien and H. Browman for editorial suggestions.

References

CONFER, J. L., AND P. I. BLADES. 1975. Omnivorous zooplankton and planktivorous fish. Limnol. Oceanogr. 20: 571−579.

CURIO, E. 1976. The ethology of predation. Springer-Verlag, New York, NY. 250 p.

EGGARS, D. M. 1982. Planktivore preference by prey size. Ecology 63: 381−390.

EVANS, B. I. 1986. Strategies and tactics of search behavior in salmonid and centrarchid planktivorous fish. Ph.D. thesis, University of Kansas, Lawrence, KS. 83 p.

EVANS, B. I., AND W. J. O'BRIEN. 1986. An analysis of the feeding rate of white crappie. Environ. Biol. Fishes. (In press)

HOLLING, C. S. 1966. The functional response of invertebrate predators to prey density. Mem. Entomol. Soc. Can. 48: 5−86.

JANSSEN, J. 1982. Comparison of searching behavior for zooplankton in an obligate planktivore, blueback herring (*Alosa aestivalis*) and a facultative planktivore, bluegill (*Lepomis macrochirus*). Can. J. Fish. Aquat. Sci. 39: 1649−1654.

LAING, J. 1938. Host-finding by insect parasites. I. Observations on the finding of hosts by *Alysia manducator*, *Mormoniella vitripennis*, and *Trichogramma evanescens*. J. Anim. Ecol. 6: 298−317.

MAINLAND, D., L. HERRERA, AND M. I. SUTCLIFFE. 1956. Statistical tables for use with binomial samples — contingency tests, confidence limits, and samples size estimates. New York University College of Medicine, New York, NY. 83 p.

O'BRIEN, W. J., AND D. I. WRIGHT. 1985. Potential limits on the daytime planktivorous feeding depth of white crappie. Verh. Int. Ver. Limnol. 22: 2527−2533.

PAINE, R. T. 1969. The *Pisaster−Tegula* interaction: prey patches, predator food preference and intertidal community structure. Ecology 50: 950−961.

PASTOROK, R. A. 1980. Selection of prey by chaoborus larvae: a review and new evidence for behavioral flexibility, p. 538−554. *In* L. C. Kerfoot [ed.] Evolution and ecology of zooplankton communities. Special Symposium Volume 3. American Society of Limnology and Oceanography, Grafton, WI.

ROSENTHAL, H., AND G. HEMPEL. 1970. Experimental studies of feeding and food requirements of herring larvae (*Clupea harengus* L.), p. 344−363. *In* J. H. Steele [ed.] Marine food chains. Oliver and Boyd, Edinburgh.

SMITH, J. N. M. 1971. Studies of the searching behavior and prey recognition of certain vertebrate predators. Ph.D. thesis, University of Oxford, Oxford.

SMITH, J. N. M., AND R. DAWKINS. 1971. The hunting behavior of individual great tits in relation to spatial variations in their food density. Anim. Behav. 19: 695–706.

WARE, D. M. 1975. Growth, metabolism, and optimal swimming speed of a pelagic fish. J. Fish. Res. Board Can. 32: 33–41.

WERNER, E. E. 1974. The fish size, prey size, handling time relation in several sunfishes and some implications. J. Fish. Res. Board Can. 31: 1531–1536.

WERNER, E. E., AND D. J. HALL. 1974. Optimal foraging and the size selection of prey by the bluegill sunfish (*Lepomis macrochirus*). Ecology 55: 1042–1052.

WRIGHT, D. I., AND W. J. O'BRIEN. 1982. Differential location of *Chaoborus* larvae and *Daphnia* by fish: the importance of motion and visible size. Am. Midl. Nat. 108: 68–73.

1984. The development and field test of a tactical model of the planktivorous feeding of white crappie (*Pomoxis annularis*). Ecol. Monogr. 54: 65–98.

Patterns in Biomass and Cover of Aquatic Macrophytes in Lakes[1]

Carlos M. Duarte, Jacob Kalff, and Robert H. Peters

Department of Biology, McGill University, 1205 Avenue Docteur Penfield, Montreal, Que. H3A 1B1

Duarte, C. M., J. Kalff, and R. H. Peters. 1986. Patterns in biomass and cover of aquatic macrophytes in lakes. Can. J. Fish. Aquat. Sci. 43: 1900–1908.

A quantitative analysis of biomass and cover of both submerged and emergent macrophytes in 139 lakes reported in the literature revealed that biomass and cover of emergent macrophytes are, on average, proportional to the lake area, but that the biomass and cover of submerged plants are proportionally reduced with increasing lake size. Underwater light was found to be the best descriptor of the cover and biomass of submerged plants. Conversely, emergent macrophytes are most strongly affected by lake morphometry, and in particular by its average slope. The predictability of the abundance of emergent and submerged macrophytes from these environmental factors stresses the predominant role that they play in macrophyte ecology and confirms the existence of strong patterns in the abundance of aquatic plants worldwide.

Une analyse quantitative de la biomasse et du couvert que représentent les macrophytes immergés et émergents de 139 lacs mentionnés dans la littérature révèlent que dans le cas des macrophytes émergents, la biomasse et le couvert sont en moyenne proportionnels à la superficie du lac, mais que dans le cas des plantes immergées, ces variables diminuent de façon inversement proportionnelle avec la superficie du lac. L'éclairage sous la surface s'est avéré le meilleur descripteur de la couverture et de la biomasse constitués par les plantes immergées. À l'inverse, les macrophytes émergents sont plus fortement affectés par la morphométrie du lac, en particulier par la pente moyenne. La prévisibilité de l'abondance des macrophytes émergents et immergés à partir de ces facteurs du milieu met en lumière le rôle prépondérant qu'ils jouent dans l'écologie des macrophytes et confirme l'existence de quasi-constantes universelles dans l'abondance des plantes aquatiques.

Received December 23, 1985
Accepted June 20, 1986
(J8620)

Reçu le 23 décembre 1985
Accepté le 20 juin 1986

Abundance of submerged macrophytes in lakes has been the subject of many regional studies (see Magnin 1893; Pearsall 1920; Maristo 1941; Spence 1967). These and other studies (reviewed by Hutchinson 1975) suggest a large number of factors that could bring about wide variation in macrophyte cover and biomass in lakes. Unfortunately, these regional studies were not designed to assess quantitatively the relative influence of each of the proposed factors on the cover and biomass of aquatic macrophytes. Data have now become available to allow a quantitative assessment of the influence of environmental factors on the abundance of lake macrophytes.

We base this assessment on two community attributes: the surface area of lakes covered by submerged and emergent macrophytes and the total biomass of these plants. Surface area covered and biomass reflect different aspects of the ways macrophytes affect their environment. Macrophyte cover influences the littoral phytoplankton and zooplankton (Hasler and Jones 1949; Goulder 1969), epiphytes (Cattaneo and Kalff 1979), littoral invertebrates (Sozska 1975), and the abundance and composition of fish (Wiley et al. 1984; Whitfield 1984). In contrast, macrophyte biomass is more closely linked to ecosystem processes such as nutrient dynamics (Carpenter 1983a; Canfield et al. 1983) and O_2 balance (Buscemi 1958; Edwards 1968).

Previous research identified light availability (Spence 1975; Chambers and Kalff 1985; Canfield et al. 1985), substrate characteristics (Pearsall 1920), lake trophic status (Hutchinson 1975), and lake morphometry (Pearsall 1917; Spence 1982; Duarte and Kalff 1986) as the most important environmental factors able to affect macrophyte cover and biomass in nature.

It is presently presumed that oligotrophic lakes tend to have lower macrophyte biomass and cover than more productive ones (i.e. Hutchinson 1975), implying that increases in lake productivity should result in higher macrophyte abundance. However, this increase should also result in decreased light penetration in the water (Canfield et al. 1983) and, subsequently, in the reduction of macrophyte cover (Phillips et al. 1978; Canfield et al. 1985). Consequently, although there is general agreement about the nature of the dominant factors influencing macrophyte cover and biomass, the effect of simultaneous changes in these variables remains unclear. Therefore, we examined available data on macrophyte cover and biomass to (1) gather empirical evidence for the relative importance and effect of the major factors on the distribution and biomass of lake macrophytes and (2) develop preliminary models to predict macrophyte distribution and biomass under a wide variety of conditions. We acknowledge that the present analysis is only a first and imprecise step in the quantification of macrophyte distribution and biomass. Yet, small steps such as this are essential beginnings in the search for pattern in nature.

[1]Contribution No. 187 to the Lake Memphremagog Project, Limnology Research Center, McGill University.

Methods

Macrophyte Characteristics

Data on macrophyte biomass and cover were obtained from the literature.[2] Macrophyte biomass is expressed in metric tons of dry weight. When other units were reported, biomass was transformed to dry weight by assuming dry weight to be 10% of fresh weight (Westlake 1965), ash free dry weight to be 80% of dry weight, and organic carbon to be 37% of dry weight (Westlake 1974). In the two cases (Lakes Marion and Kalgaard) where only productivity values were provided for submerged macrophytes, biomass was assumed to correspond to two thirds of production (Rich et al. 1971). Surface area covered by macrophytes is expressed in hectares. When only vegetation maps were provided, macrophyte cover was determined by planimetry, using a Hi-Pad digitizer (Houston Instruments).

Environmental Variable Selection

Light availability, sediment characteristics, lake trophy, and lake morphometry are believed to be the major determinants of macrophyte cover and biomass. However, no single paper contains measures of all the environmental data needed for a given lake. We therefore used an estimate of the underwater light climate to reflect both photosynthetically available radiation and lake trophy. The underwater light climate is partially a function of geographic location of the lake because location determines both the incident radiation and whether it is reduced by a winter ice cover. Trophy and water color, in turn, determine light extinction in the water column. The needed annual irradiance at the lake surface (I_a, kilocalories per square centimetre per year) was obtained from climatic maps (Landsberg et al. 1966), and the annual amount of light penetrating the water surface was derived from existing relationships (Appendix). Additional climatic information such as the mean annual precipitation (P, millimetres per year) was obtained from a hydrological atlas (Kouzoun et al. 1977).

The proportion of the total amount of light available to submerged macrophytes depends on the depths at which the plants grow and the absorption of light in the water column. To represent this, we developed composite variables to describe an average underwater irradiance. The amount of I_0 remaining at depth Z in the water column ($\bar{I}_z$) depends on the coefficient of attenuation, k, as defined by the Beer–Lambert law: $I_z = I_0 e^{-kZ}$. The irradiance received by rooted plants is dependent on the irradiance received at the lake bottom. This was estimated by calculating the annual irradiance received down to the mean depth of the lake ($I_{\bar{Z}}$). However, as macrophytes grow towards the lake surface, they depend more on the average light intensity in the water column ($\bar{I}_{\bar{Z}}$) than on the light received at the bottom. The average irradiance within the mean depth of the lake was calculated using Riley's (1957) integration of the Beer–Lambert law: $\bar{I}_{\bar{Z}} = I_0 (1 - k\bar{Z}) / (k\bar{Z})$. When k was not provided, it was approximated from mean summer Secchi depth (S_d) assuming $k = 1.46/S_d$ (Walker 1982). Mean summer Secchi depth was obtained from the same source as the macrophyte data or from the literature there mentioned, but only if the measurements were made within 1 yr of the macrophyte survey. Water colour (milligrams Pt per litre) for the Finnish data used was transformed to Secchi depth by using the equation developed by Eloranta (1978): $S_d = 17.54\ \text{colour}^{-0.57}$. These calculations idealize the lake as a straight-sided water body with the same surface as the real lake but with a constant depth, $\bar{Z}$. The two variables representing underwater irradiance ($I_{\bar{Z}}$ and $\bar{I}_{\bar{Z}}$) reflect the major factors affecting the underwater light climate by combining the theoretical effects of lake morphometry ($\bar{Z}$), trophy and water colour (through the attenuation coefficients, k), and geographical position (through its effect on irradiance) into single variables.

Since sediment characteristics and wave action depend on lake morphometry (Hakanson 1981), we used morphometric factors as indexes of the sediment type and the energy environment of the littoral zone. Lake area (A) was used to scale the lakes with respect to their total cover and biomass of submerged plants and as an index of wave action. The steepness of the lake basin was characterized by the index $S = \sqrt{A}/\bar{Z}$ (Hakanson 1981), and the shape of the basin represented by the ratio of mean to maximum depth ($\bar{Z}/Z_{max}$) (Carpenter 1983b).

TABLE 1. Mean, range, and number of observations for the different measures of macrophyte cover and biomass and the environmental parameters correlated with them. A = lake area; $\bar{Z}$ = mean depth; Z_{max} = maximum depth; S_d = Secchi depth; I_a = annual surface irradiance; Lat. = latitude; P = annual precipitation; L_{ic} = length of ice cover; $\%A_m$ = percent lake area covered by macrophytes; $\%A_s$ = percent lake area covered by submerged plants; $\%A_e$ = percent lake area covered by emergent macrophytes; R_{bs} = biomass of submerged plants per unit area colonized; R_{be} = biomass of emergent plants per unit area colonized.

Variable	Mean	Maximum	Minimum	No. of observations
A (ha)	194	2.48×10^6	0.038	139
Z_{max} (m)	21.8	310.0	1.0	130
$\bar{Z}$ (m)	7.7	152.0	0.2	116
S_d (m)	2.8	16.0	0.3	102
I_a (kcal·cm^{-2}·yr^{-1})	109	200	70	139
Lat. (degrees)	46.0	69.0	1.0	139
P (mm·yr^{-1})	1150	3200	420	139
L_{ic} (days)	93	288	0	139
$\%A_m$ (%)	44.1	100.0	0	104
$\%A_s$ (%)	35.0	100.0	0	82
$\%A_e$ (%)	12.3	100.0	0	77
R_{bs} (t dry wt·ha^{-1})	3.1	24.6	0.021	51
R_{be} (t dry wt·ha^{-1})	15.1	120.0	0.13	28

Statistical Analysis

Relationships between macrophyte cover or biomass and the various measures of light, trophy, and morphometry were described by regression analysis. These variables were transformed, if necessary, to meet statistical requirements of linear regression, following Box and Tidwell (1962), and a stepwise procedure was used to select the best independent variables for each model. The significance of the coefficients were tested by t-test, and the relative contributions of the different regressors in multivariate models were determined by ANOVA (Moesteller and Tukey 1977). The standard error of the estimates (SE_{est}) were calculated to indicate the error involved in the models. The predictive power of the models was assessed by cross-validation (Draper and Smith 1966). For this purpose

[2]A complete data set and reference list are available, at a nominal cost, from the Depository of Unpublished Data, CISTI, National Research Council of Canada, Ottawa, Canada K1A 0S2.

TABLE 2. Correlation matrix between the climatic and limnological factors used as regressors of macrophyte abundance. Only values with $P < 0.05$ are shown. S = slope; $I_{\bar{z}}$ = annual irradiance at the mean depth. Other symbols as in Table 1.

	A^a	$\bar{Z}$	Z_{max}	S	S_d	I_a	Lat.	P	L_{ic}	$I_{\bar{z}}$
A	—	0.41	0.37	0.70	0.22	0.28	−0.29	0.22	−0.22	0.49
$\bar{Z}$		—	0.94	0.20	0.58	—	—	—	—	—
Z_{max}			—	0.20	0.60	—	—	—	—	—
S				—	0.28	−0.32	0.25	—	—	−0.31
S_d					—	—	—	0.35	—	—
I_a						—	−0.88	0.45	−0.80	0.83
Lat.							—	−0.58	0.80	0.78
P								—	−0.62	0.45
L_{ic}									—	0.80
$I_{\bar{z}}$										—

[a]Log-transformed before correlations were calculated.

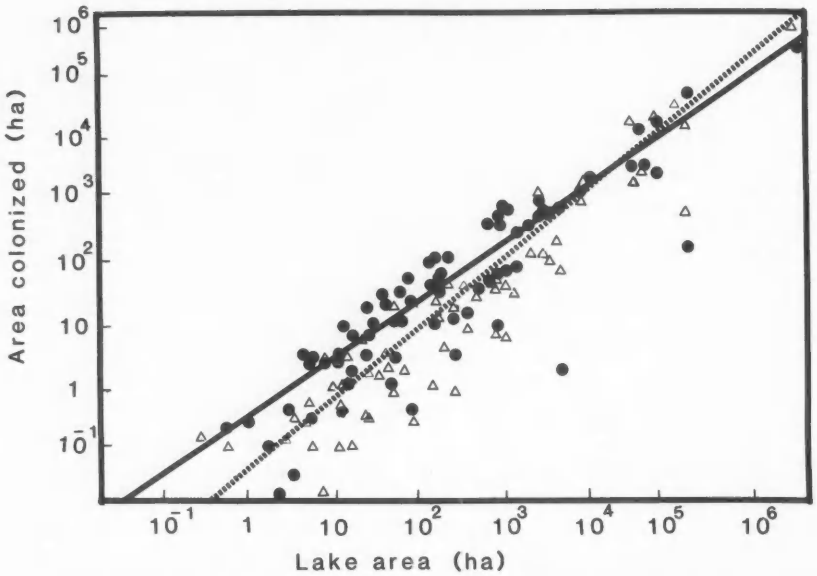


FIG. 1. Relationship between the surface area covered by submerged (●) and emergent (△) macrophytes and lake area. Regression lines for submerged (Equation 1, Table 3) and emergent (Equation 8, Table 4) plants are shown as a continuous and broken line, respectively.

10% of the observations in the data set were randomly selected and the predictions for these obtained from the regression built with the rest of the data set. The procedure was iterated until the number of observations tested equaled approximately 50% of the total data set. The accuracy of the predictions can then be judged by plotting the observed values for each set of selected points against the values predicted by the regressions built with the remaining observations. The results can be assesed by comparing them with statistics used for evaluating the fit (R^2 and SE_{est}).

Data Set

The data set included 139 lakes and covered a wide range of values for all variables (Table 1). However, not all variables were measured in all lakes, and therefore the number of observations decreased significantly when multivariate models were attempted. The majority of the lakes were located in Europe (58) or North America (62), but lakes in Africa (6), South Africa (4), Asia (6), and Australia (1) were included. The lakes ranged in latitude from Lake Curua−Una, Brazil (latitude 3°S) to subantartic Lakes Sombre, Moss, and Changing (latitude 61°S). The size varied from 2.5×10^6 ha (Lake Chad) to small ponds such as Borax lake, California (0.038 ha); maximum depth ranged from 310 m in Loch Morar, Scotland, to 1 m in some oxbow lakes, Alberta. Secchi depths varied between 0.3 m in extremely turbid lakes like Lake Chilwa, Malawi, to 16 m in clear lake Leman (France−Switzerland). Low Secchi readings were generally attributable to high algal biomass; however, it cannot be taken strictly as an index of trophy because coloured lakes, generally oligotrophic, also have high extinction coefficients and because low Secchi depths may also be attributable to sediment resuspension as in some shallow large lakes (i.e. Neusiedlersee, Austria). Salinity varied from freshwater to seawater in some coastal lakes, and the data set included 11 lakes with conductivities above 1000 $\mu S \cdot cm^{-1}$

TABLE 3. Regression models describing the total (A_s) and the percent lake surface ($\%A_s$) colonized by submerged macrophytes, their total biomass (B_s), and their biomass per unit area colonized (R_{bs}) as a function of environmental factors. The relationship between biomass and area colonized is also shown. The variable S_{al} takes values 1 or 0 depending on whether the lake has a conductivity above or below 1000 $\mu S \cdot cm^{-1}$. Other symbols and units as in Table 1.

Equation	R^2	N	P	$SE_{ln\ est}$
(1) $\ln A_s = 0.91 \ln A - 1.1$	0.80	76	<0.0001	1.47
(2) $\ln B_s = 0.89 \ln A - 1.42$	0.59	70	<0.0001	3.04
(3) $\ln A_s = 0.94 \ln A + 0.85 \ln I_{\bar{z}} - 3.7$	0.89	51	<0.0001	1.05
(4) $\ln B_s = 0.95 \ln A + 1.12 \ln I_{\bar{z}} - 4.6$	0.66	56	<0.0001	2.14
(5) $\ln B_s = 0.99 \ln A_s + 0.37$	0.84	51	<0.0001	1.26
(6) $\sqrt{R_{bs}} = 0.06\sqrt{P} + 0.05 I_{\bar{z}}^2 - 5.6 \times 10^{-4} I_{\bar{z}} - 1.3$	0.51	39	<0.0001	0.77[a]
(7) $\%A_s = 1.4 I_{\bar{z}} + 0.07 L_{ic} - 24 S_{al} - 0.90$	0.60	55	<0.0001	11.2[b]

[a] SE_{est} for R_{bs} is the standard error of the square root of the predicted value.
[b] SE_{est} for $\%A_s$ is the standard error of the untransformed percentage.

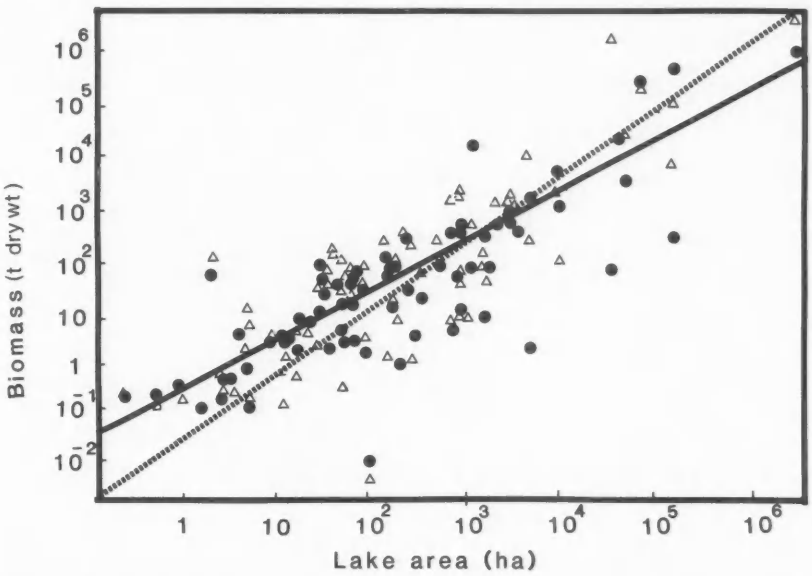


FIG. 2. Relationship between lake size and the biomass of submerged (●) and emergent (△) macrophytes. Regression lines for submerged (Equation 2, Table 3) and emergent (Equation 11, Table 4) plants are shown as a continuous and broken line, respectively.

Results and Discussion

The climatic and morphometric variables that were correlated with macrophyte biomass and cover were not redundant in that the correlation coefficients among them were not very high (Table 2).

Submerged Macrophytes

Both total area covered and total biomass will tend to be greater in larger lakes, but there is no a priori reason to expect that any given standardization (e.g. division by lake area) will stabilize this effect. Consequently, the effect of lake size must be considered before any other factor can be effectively analyzed.

The analysis showed that the surface area covered by submerged macrophytes (A_s) is not a constant proportion of the lake area (A) (Fig. 1), but tends to be smaller in bigger lakes (H_o: exponent = 1, $P < 0.05$; Equation 1, Table 3). The equation for submerged biomass (B_s, Equation 2, Table 3) indicates that, per unit area, it too is smaller in bigger lakes (H_o: exponent = 1, $P < 0.10$; Fig. 2). Thus, the relative importance of submerged macrophytes will be, on average, higher in smaller lakes. The reason for the relative reduction of submerged macrophytes in larger lakes is partially related to the positive correlation between lake size and mean depth ($R^2 = 0.41$, $P < 0.01$), resulting in larger areas below the depth of maximum penetration of submerged plants.

The analysis indicates that cover is not merely a function of lake area, but that submerged plants cover a larger proportion of lakes receiving more irradiance (Equation 3, Table 3). The underwater irradiance is the single most significant correlate of submerged plant cover and explained a large proportion of the variance. Cross-validation of the model shows that the equation has considerably predictive power ($R^2 = 0.81$, $SE_{ln\ est} = 0.99$). The magnitude of the deviations from the calculated values

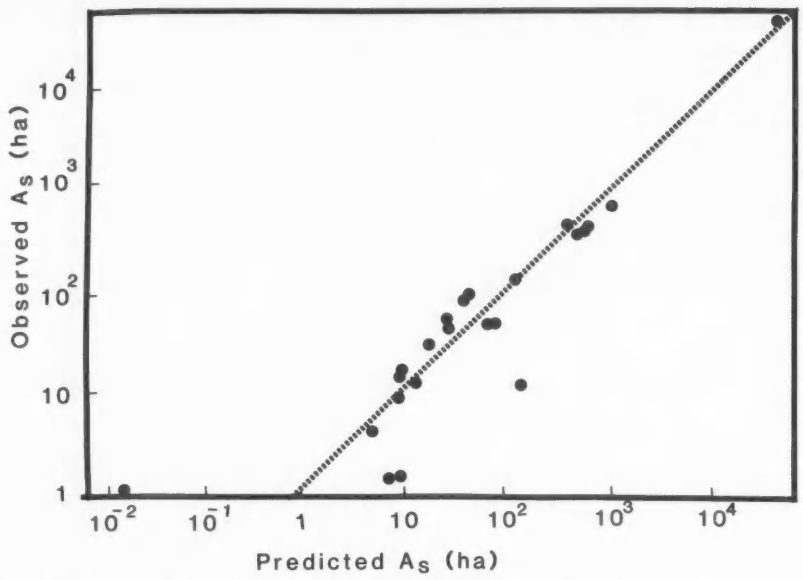


FIG. 3. Cross-validation of the model predicting submerged cover (Equation 3, Table 3) showing the relationship between the values predicted from the model and observed values for independent sets of data.

(Fig. 3) show that the uncertainty around the prediction for the area covered is approximately an order of magnitude. Although this is not very precise, the precision is comparable with that of phytoplankton models widely used in lake ecology and management (Dillon and Rigler 1974; Vollenweider and Kerekes 1982) and should, therefore, be of equivalent value. It is, in any case, the only such relationship available.

An analysis of the effect of various environmental factors on submerged biomass again revealed biomass to increase with increasing underwater irradiance (Equation 4, Table 3). The relationship between submerged biomass and irradiance was, however, weaker than that for cover and was not improved by the consideration of other factors. While the low resolution of the equation prevents its use for predicting macrophyte biomass, it also shows a pattern, providing additional evidence for the dominant role of light availability on the ecology of submerged macrophytes. However, submerged biomass can be estimated with greater precision (cross-validation $R^2 = 0.88$, $SE_{\ln\ est} = 1.49$) if the area covered by submerged plants is known (Equation 5, Table 3).

The overwhelming importance of light availability in determining the cover and biomass of submerged macrophytes (Equations 3 and 4, Table 3) implies that long-term changes in underwater irradiance should lead to parallel changes in submerged cover and biomass at any one site. Such changes can result from changes on (1) climate (that determines the incoming irradiance), (2) the transparency of the water (determining the attenuation of irradiance on the water column), and (3) the depth of the lake (which determines the extent of the attenuation of irradiance before reaching the bottom). From our approximation of the underwater irradiance ($I_Z = I_o e^{-z1.46/S_d}$), it is evident that changes in incoming irradiance will result in a smaller response of the submerged macrophyte cover than if the water transparency (S_d) or mean depth of the lake ($\bar{Z}$) changes. This may well account for the absence of literature evidence for the effect of latitude on cover and biomass of submerged plants and explain, in contrast, why reductions in water transparency have long been associated with a submerged macrophyte decline (Phillips et al. 1978; Ozimek and Kowalczewski 1984). The scatter in the relationship between submerged biomass and the area the plants cover is relatively large (Equation 5, Table 3), suggesting that the biomass of submerged plants per unit area colonized is not constant. The irradiance received by the submerged plants has been postulated to determine their maximum biomass (C. M. Duarte and J. Kalff, unpubl. data). We therefore tested whether differences in underwater irradiance account for the observed variability in submerged biomass per unit area colonized. Regression analysis supported this (Equation 6, Table 3), indicating that well-irradiated lakes support a greater mean submerged biomass per unit area colonized than those with reduced underwater irradiance. However, the increase in biomass with increasing underwater irradiance is not constant, but appears to decrease with increasing light levels as reflected in the negative $I_{\bar{Z}}^2$ term (Equation 6, Table 3). In addition to irradiance, submerged biomass was also positively related to the annual precipitation. This pattern is similar to the positive relationship proposed for phytoplankton (Goldman and Amezaga 1984) and suggests that while the precipitation may represent a small proportion of the total nutrient loading, the fact that it is more evenly distributed over the lake surface may make it more amenable to enhance plant growth.

The large effect of lake size on cover could overshadow the relationships between cover and environmental factors other than irradiance. These factors might therefore be better examined by using the percent lake area covered by submerged macrophytes as the appropriate dependent variable (Equation 7, Table 3). The results support the strong positive relationship between cover ($\%A_s$) and the mean irradiance within the mean depth of the lake ($I_{\bar{Z}}$). An analysis of covariance with a dummy variable that discriminates lakes with conductivities above and

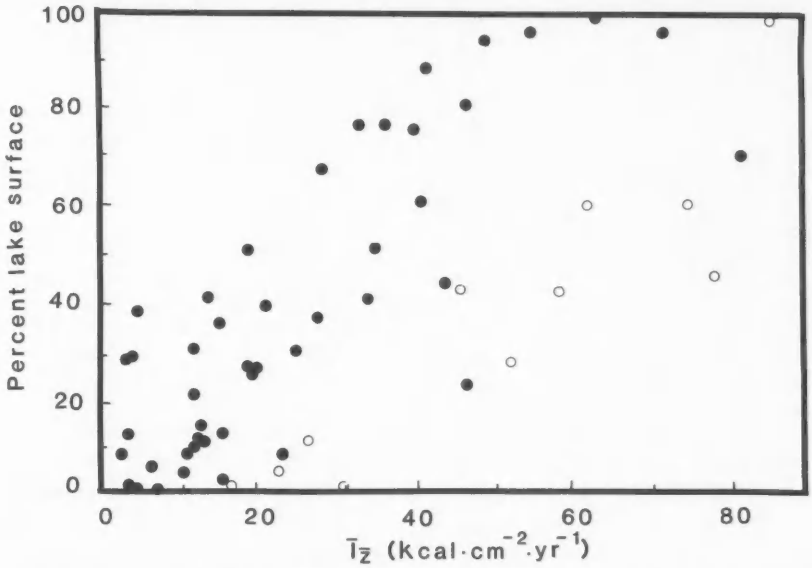


FIG. 4. Percent lake area covered by submerged macrophytes as a function of the mean irradiance within the mean depth of the lake. ○, highly saline lakes (>1000 μS·cm^{-1}).

TABLE 4. Models relating the total area (A_e) and percent area ($\%A_e$) colonized by emergent macrophytes and the total biomass (B_e) and biomass per unit area (R_{be}) colonized by emergent macrophytes to environmental factors. Symbols and units as in Table 1.

Equation	R^2	N	P	$SE_{ln\ est}$
(8) $\ln A_e = 1.0 \ln A - 2.6$	0.87	60	<0.0001	1.33
(9) $\ln A_e = 0.72 \ln A - 0.69 \ln S + 0.72$	0.90	60	<0.0001	1.28
(10) $\%A_e = 2.81 \ln A - 0.21\bar{I}_{\bar{Z}} - 6.58 \ln Z_{max}$	0.41	53	<0.0001	11.59[a]
(11) $\ln B_e = 1.1 \ln A - 1.4$	0.70	36	<0.0001	2.53
(12) $\ln B_e = 0.57 \ln A - 1.34S + 4.77$	0.76	32	<0.0001	2.47
(13) $\ln B_e = 1.17 \ln A_e + 0.89$	0.91	28	<0.0001	1.45
(14) $R_{be} = 4.93 \ln A + 0.75\text{lat.} + 62.5(\bar{Z}/Z_{max}) - 10 \ln \bar{Z} - 6$	0.52	26	<0.002	17.0[b]

[a] SE_{est} of $\%A_e$ in percentage.
[b] SE_{est} of R_{be} in t dry wt·ha^{-1}.

below 1000 μS·cm^{-1} further suggests that submerged macrophytes cover a smaller proportion of the area of more saline lakes (Fig. 4), a finding supported by numerous observations. Williams (1978) noted that submerged macrophyte development in lakes is severely affected by salinity, and Hammer (1981) mentioned that submerged macrophytes are less abundant in saline lakes. Since some of these observations refer to noncoastal saline lakes, the significant factor appears to be salinity, not vicinity to the sea.

Emergent Macrophytes

The relationship between lake size on the one hand and area and biomass of emergent macrophytes on the other also needs to be examined before assessing the effect of environmental factors on cover and biomass. The relationship between the area colonized (A_e) and lake size appears to be linear (H_o: exponent = 1, $P = 0.74$; Equation 8, Table 4). This indicated that emergent macrophytes colonize, on average, 7% of the lake area regardless of the size of the lake, and because submerged plants cover a smaller area in larger lakes, emergent cover will become more important with increasing lake size (Fig. 1). The constancy of the fraction of lake area covered by emergent plants was also noted for Polish lakes (Planter 1973), where the area covered by emergent plants varied between 9.3 and 12.3%. In contrast, Spence (1982) postulated that the area colonized by the emergent plants should decline with increasing lake area as a consequence of increased wave action in the littoral. The absence of such a decline here is probably attributable to the increased number of sheltered bays and floodplains in larger lakes, which compensate for the increased wave effect postulated. The relationship between emergent cover and lake area was improved by considering also the steepness of the basin (Equation 9, Table 4), probably because steep-sided lakes have a smaller area suitable for emergent growth. Furthermore, the percent lake area covered by the plants ($\%A_e$) is not constant but is greater in larger lakes and smaller in deep lakes and lakes receiving high irradiance (Equation 10, Table 4).

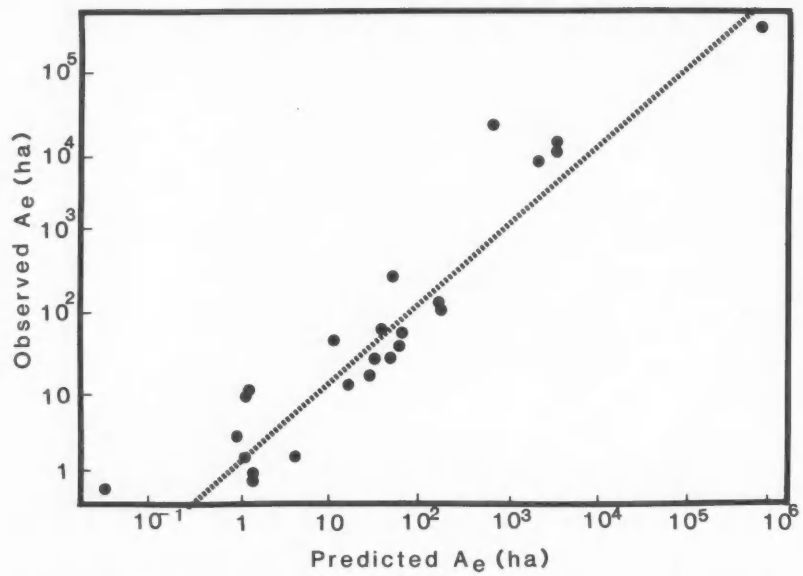


FIG. 5. Agreement between the predicted cover of emergent macrophytes (Equation 9, Table 4) and independent values of observed cover obtained by cross-validation.

The biomass of emergent plants seems to increase linearly with lake size (H_o: exponent = 1, $P = 0.57$; Equation 11, Table 4), and, again as a rough approximation, our analysis indicates that this biomass is about 0.25 t dry wt·ha^{-1}, the variability in the relationship between emergent biomass and lake size being smaller than that in the analogous one for submerged plants. Biomass of emergent macrophytes also increases more rapidly with lake size than the biomass of submerged ones and is consequently normally greater in larger lakes (Fig. 2). Equation 12 (Table 4) suggests that emergent biomass also declines with increased steepness of the lake basin. The equation relating emergent macrophytes biomass to the surface they cover (Equation 13, Table 4) has a particular good fit ($R^2 = 0.91$, $SE_{\ln\ est} = 1.4$) and can be used to estimate the biomass of emergent macrophytes with better than an order of magnitude resolution (cross-validation $R^2 = 0.93$, $SE_{\ln\ est} = 1.4$; Fig. 5). When the variation in biomass of emergent plants per unit area colonized was analyzed, we found it to be greater in larger and in shallower lakes and to be positively related to latitude and the depth ratio ($\bar{Z}/Z_{max}$), indicating that pan-shaped lakes support a higher biomass of emergent plants than cone-shaped ones, with steeper slopes (Equation 14, Table 4).

Our models show that the biomass and area colonized by submerged plants are primarily affected by the underwater light regime, as determined by lake morphometry, by the extinction coefficient, and by latitude. In contrast, the total biomass and cover of emergent plants appear to be unaffected by the available irradiance but are affected by lake morphometry, since both biomass and cover are related to the steepness and form of the basin and the lake area.

While we considered submerged and emergent macrophytes as separate entities in the analysis, it is clear that they are highly integrated, forming a continuous cover in the littoral zone. Conceivably, the surface area covered by emergent and submerged plants together may be more precisely related to environmental variables than emergent or submerged cover separately. This was indeed the case, because the total cover of macrophytes (A_m, hectares) is positively related to the annual irradiance at the surface (I_a, kilocalories per square centimetre per year), the annual irradiance at the mean depth of the lake ($I_{\bar{z}}$, kilocalories per square centimetre per year), and the period of ice cover (L_{ic}, days), the equation being

$$\ln A_m = 0.97 \ln \text{area} + 0.98 \ln \bar{I}_{\bar{z}} + 0.009\, L_{ic} + I_a,$$
$$R_2 = 0.93,\ N = 71,\ P < 0.0001,\ SE_{\ln\ est} = 0.86.$$

The model is more precise than either the ones describing the emergent or the submerged cover, respectively (cross-validation $R^2 = 0.94$, $SE_{\ln\ est} = 0.71$). The scatter around the predicted cover indicates that predictions from this equation are within half an order of magnitude from the observed value (Fig. 6). This indicates that although submerged and emergent macrophytes are affected by different factors in the environment, their separation entails a significant loss of information because they form a continuum in the littoral zone. The relationship obtained suggests that the total macrophyte cover as a whole is largely determined by the underwater light regime and the annual surface irradiance, the latter providing a better indication of the light available for growth of emergent plants. The observed relationship between the length of ice cover and the total area covered by macrophytes suggests that, once the effect of reduced irradiance ($I_{\bar{z}}$) has been corrected for, the stable conditions provided by an environment devoided of wave action may enable plants to remain overwinter, without necessarily resulting in increased productivity. In contrast with cover, a consideration of both emergent and submerged macrophytes as a whole did not result in more precise estimates of the total macrophyte biomass, probably because the biomass per unit area is on average one order of magnitude higher for emergent than for submerged macrophytes.

The models describing macrophyte cover are more precise than those for biomass. This may imply a stronger link between surface cover and the factors we studied but could equally well

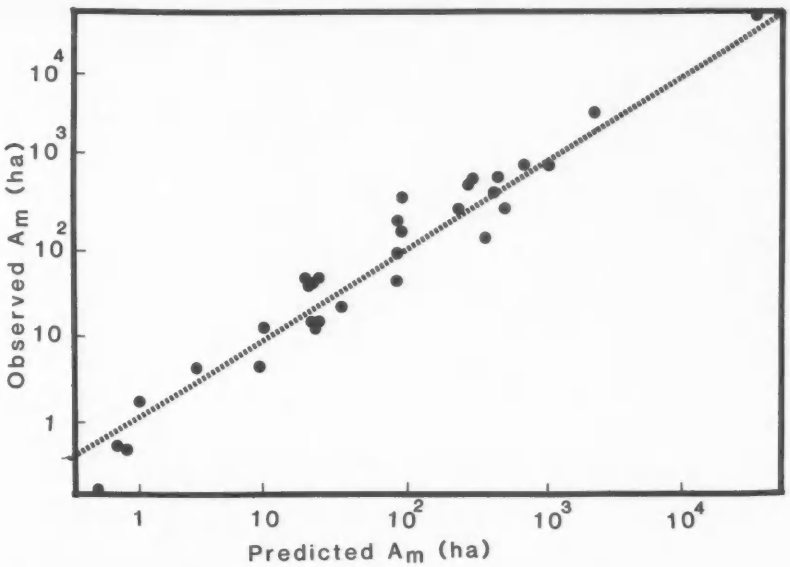


FIG. 6. Cross-validation of the model predicting total macrophyte cover (Equation 15) showing the values predicted for independent observations.

be the result of the greater patchiness of macrophyte biomass within lakes resulting from the physical heterogeneity of the littoral zone. The latter would influence the mean lake values used in our analysis and results in the greater uncertainity associated with the estimation of the submerged biomass in particular. Nevertheless, the present analysis reveals consistent patterns in macrophyte cover and biomass and provides the first empirical models for (1) the prediction of the total surface area covered by macrophytes, (2) the area covered by submerged and emergent plants from simple climatic and morphometric factors, respectively, and (3) the prediction of the macrophyte biomass from the more easily estimated area of cover.

References

BOX, G. E. P., AND P. H. TIDWELL. 1962. Transformation of the independent variables. Technometrics 4: 531–540.

BUSCEMI, P. A. 1958. Littoral oxygen depletion produced by a cover of *Elodea canadensis*. Oikos 9: 239–245.

CANFIELD, D. E. JR., K. A. LANGELAND, S. B. LINDA, AND W. T. HALLER. 1985. Relations between water transparency and maximum depth of macrophyte colonization in lakes. J. Aquat. Plant Manage. 23: 25–28.

CANFIELD, D. E. JR., K. A. LANGELAND, M. J. MACEINA, W. T. HALLER, J. V. SHIREMAN, AND J. R. JONES. 1983. Trophic state classification of lakes with aquatic macrophytes. Can. J. Fish. Aquat. Sci. 40: 1713–1718.

CARPENTER, S. R. 1983a. Submersed macrophyte community structure and internal loading: relationship to lake ecosystem productivity and succession, p. 105–111. *In* J. Taggart [ed.] Lake restoration, protection and management. U.S. EPA, Washington, DC.

1983b. Lake geometry: implications for production and sediment acretion rates. J. Theor. Biol. 105: 273–286.

CATTANEO, A., AND J. KALFF. 1979. Primary production of algae growing on natural and artificial aquatic plants: a study of interactions between epiphytes and their substrate. Limnol. Oceanogr. 24: 1031–1037.

CHAMBERS, P. A., AND J. KALFF. 1985. Depth distribution and biomass of submersed macrophyte communities in relation to Secchi depth. Can. J. Fish. Aquat. Sci. 42: 701–709.

DILLON, P. J., AND F. H. RIGLER. 1974. The phosphorus–chlorophyll relationship in lakes. Limnol. Oceanogr. 19: 767–773.

DRAPER, N. R., AND H. SMITH. 1966. Applied regression analysis. John Wiley & Sons, New York, NY.

DUARTE, C. M., AND J. KALFF. 1986. Littoral slope as a predictor of the maximum biomass of submerged macrophyte stands. Limnol. Oceanogr. (In press)

EDWARDS, R. W. 1968. Plants as oxygenators in rivers. Water Res. 2: 243–248.

ELORANTA, P. 1978. Light penetration in different types of lakes in Central Finland. Holarct. Ecol. 1: 362–366.

GOLDMAN, C. R., AND E. DE AMEZAGA. 1984. Primary productivity and precipitation at Castle Lake and Lake Tahoe during twenty-four years, 1959–1982. Verh. Int. Ver. Limnol. 22: 591–599.

GOULDER, R. 1969. Interactions between the rate of production of freshwater macrophytes and phytoplankton in a pond. Oikos 20: 300–309.

HÅKANSON, L. 1981. Bottom dynamics in lakes. Hydrobiologia 91: 9–22.

HAMMER, V. T. 1981. Primary production in saline lakes. Hydrobiologia 81: 47–57.

HASLER, A. D., AND E. JONES. 1949. Demonstration of the antagonistic action of large aquatic plants on algae and rotifers. Ecology 30: 359–364.

HUTCHINSON, G. E. 1975. A treatise on limnology. Vol. III. Limnological botany. John Wiley & Sons, New York, NY. 660 p.

KOUZOUN, V. I., A. A. SOLOKOV, M. I. BUDYKO, K. P. VOSKRESENSKY, G. P. KALININ, A. A. KONOPLYANSTSER, E. S. KOROTKEVICH, AND M. I. LVOVICH [ED.]. 1977. Atlas of world water balance. Hydrometeorological Publishing House, UNESCO, Paris.

LANDSBERG, H. E., H. LIPPMANN, K. H. PAFFEN, AND C. TROLL. 1966. World maps of climatology. Springer-Verlag Publ., Berlin. II + 19 p.

MAGNIN, A. 1893. Recherches sur la vegetation des lacs du Jura. Rev. Gen. Bot. 5: 241–257, 303–316.

MARGALEF, R. 1984. Limnologia. Omega, Madrid. 1010 p.

MARISTO, L. 1941. Die seetypen Finnlando auf floristracher und vegetationsphysiognomischer. Grundlage. Ann. Soc. Zool. Bot. Vanamo 15.n'5.

MOESTELLER, F., AND J. W. TUKEY. 1977. Data analysis and regression. A second course in statistics. Addison-Wesley Publishing Co., Reading, MA. XVII + 585 p.

OZIMEK, T., AND A. KOWALCZEWSKI. 1984. Long-term changes of the submerged macrophytes in eutrophic Lake Mikolajskie (North Poland). Aquat. Bot. 9: 1–11.

PEARSALL, W. H. 1917. The aquatic and marsh vegetation of Esthwaite water. J. Ecol. 5: 180–201.

1920. The aquatic vegetation of the English lakes. J. Ecol. 8: 163–201.

PHILLIPS, S. L., D. EMINSON, AND B. MOSS. 1978. A mechanism to account for macrophyte decline on progressively eutrophicated freshwaters. Aquat. Bot. 4: 103–126.

PLANTER, M. 1973. Physical and chemical conditions in the helophyte zone of the lake littoral. Pol. Arch. Hydrobiol. 20: 1–7.

RICH, R. H., R. G. WETZEL, AND VAN THUY. 1971. Distribution, production and role of aquatic macrophytes in a southern Michigan marl lake. Freshwater Biol. 1: 3–21.

RILEY, G. A. 1957. Phytoplankton of the North Central Sargasso Sea, 1950–52. Limnol. Oceanogr. 2: 252–270.

SHUTER, B. J., D. A. SCHLESINGER, AND A. P. ZIMMERMAN. 1983. Empirical prediction of annual surface water temperature cycles in North American lakes. Can. J. Fish. Aquat. Sci. 40: 1838–1845.

SMITHSONIAN INSTITUTE. 1939. Smithsonian meteorological tables. Smithsonian Institute, Washington, DC. 239 p.

SOZSKA, G. J. 1975. Ecological relations between invertebrates and submersed macrophytes in the lake littoral. Ekol. Pol. 23: 393–415.

SPENCE, D. H. N. 1967. Factors controlling the distribution of freshwater macrophytes with particular reference to the lochs of Scotland. J. Ecol. 8: 163–204.

1975. Light and plant response in freshwater, p. 93–133. *In* G. C. Evans, R. Bainbridge, and O. Rackmam [ed.] Light as an ecological factor. II. Blackwell Scientific Publications, London.

1982. The zonation of plants in freshwater lakes. Adv. Ecol. Res. 12: 37–125.

STRASKRABA, M. 1980. The effect of physical variables on freshwater production: analysis based on models, p. 13–84. *In* E. D. Le Cren and R. H. Lowe-McConnell [ed.] The functioning of freshwater ecosystems. Cambridge University Press, Cambridge.

VOLLENWEIDER, R. A., AND J. J. KEREKES. 1982. Eutrophication of waters. Monoriting, assessment, and control. OECD, Paris. 154 p.

WALKER, T. A. 1982. Use of a secchi disc to measure attenuation of underwater light for photosynthesis. J. Appl. Ecol. 19: 539–544.

WESTLAKE, D. F. 1965. Theoretical aspects of the comparability of productivity data. Mem. Ist. Ital. Idrobiol. 18 (Suppl.): 313–322.

1974. Sampling techniques and methods for estimating quantity and quality of biomass (macrophytes), p. 32–41. *In* R. A. Vollenweider [ed.] A manual of methods for measuring primary production in aquatic environments. I.B.P. Handb. No. 12. Blackwell Scientific Publications, Oxford. 225 p.

WHITFIELD, A. K. 1984. The effects of prolonged aquatic macrophyte senescence on the biology of the dominant fish species in a southern African coastal lake. Estuarine Coastal Shelf Sci. 18: 315–329.

WILEY, J. L., R. W. GORDEN, S. W. WAITE, AND T. POWLESS. 1984. The relationship between aquatic macrophytes and fish production in Illinois ponds: a simple model. N. Am. J. Fish. Manage. 4: 111–119.

WILLIAMS, W. D. 1978. Inland waters, p. 19–40. *In* H. A. Nix and M. A. Elliott [ed.] Managing aquatic ecosystems. Proc. Ecol. Soc. Aust. Vol. 8.

Appendix

Incoming irradiance penetrates open water and ice differently. Therefore, a correction for this is necessary. When the length of ice cover in days (L_{ic}) was not specified, we used the equation developed by Shuter et al. (1983):

$$\ln (L_{ic}) = 360 - 0.06T + 0.073 \ln (\bar{Z}) + 5.0$$

where T is the mean annual temperature (degrees Celsius) and $\bar{Z}$ is the mean depth of the lake. The mean annual temperature was calculated from the equation (Straskraba 1980)

$$T = 25.9 + 4.89 \times 10^{-1} \text{ lat.} - 2.74 \times 10^{-2} \text{ Lat.}^2 + 1.78 \times 10^{-4} \text{ lat.}^3$$

When only the maximum depth of the lake was available, the mean depth of the lake was calculated from an equation we developed for lakes for which both were reported:

$$\bar{Z} = 0.4\, Z_{max} - 0.54, \quad R^2 = 0.89,\ N = 102,\ P < 0.0001.$$

This estimation of mean depth was used only to calculate the length of ice cover. Once the L_{ic} was estimated, we took the average reflectances of an ice cover surface and open water to be 90 and 10%, respectively (Margalef 1984). Consequently, the total irradiance just under the water surface (I_o) can be approximated as

$$I_o = 0.9I_s + 0.1I_i$$

where I_s is the irradiance during the ice-free season and I_i is that reaching the ice-covered lake; 0.9 and 0.1 represent the proportion of incident light which is not reflected by water and ice, respectively. The irradiances reaching the lake while ice covered (I_i) and ice free (I_s) were obtained from the total annual irradiance (I_a) and the calculated length of the ice cover (L_{ic}) (Shuter et al. 1983), assuming that one third of the L_{ic} occurs before the winter solstice and two thirds thereafter. The relation describing the percentage of the annual irradiance ($\%I = 100^* I_i/I_o$) received on each Julian day (D) at different latitudes (lat.) was developed from data in the Smithsonian tables (Smithsonian Institute 1939):

$$\%I = 19.3 + 26 \times 10^{-4} D^2 - 0.24 \text{ lat.} - 15 \times 10^{-4} \text{ lat.}^2, \quad R^2 = 0.96,\ N = 120,\ P < 0.0001.$$

The equation was used with the value of I_a for each lake to calculate I_s, I_i, and finally I_o.

Environmental Factors Regulating the Seaward Migration of European Silver Eels (*Anguilla anguilla*)

L. A. Vøllestad[1]

Department of Biology, Division of General Physiology, University of Oslo, P.O., Box 1051, Blindern, N-0316 Oslo 3, Norway

B. Jonsson, N. A. Hvidsten, and T. F. Næsje

Directorate for Nature Management, Fish Research Division, Tungasletta 2, N-7000 Trondheim, Norway

Ø. Haraldstad

Vest-Agder County Department of the Environment, Tinghuset, Holbergsgt. 54, N-4600 Kristiansand S, Norway

and J. Ruud-Hansen

Centre for Experimental Design and Data Processing, N-1432 Ås-NLH, Norway

Vøllestad, L. A., B. Jonsson, N. A. Hvidsten, T. F. Næsje, Ø. Haraldstad, and J. Ruud-Hansen. 1986. Environmental factors regulating the seaward migration of European silver eels (*Anguilla anguilla*). Can. J. Fish. Aquat. Sci. 43: 1909–1916.

We examined the connection between descent of European silver eels (*Anguilla anguilla*) and water discharge, water temperature, turbidity, photoperiod, light intensity, and moon phase based on 10 yr of field data and field experiments. The eel migration takes place between August and December, and mean water temperature for July–August and mean water discharge for August–October explained 91% of the total variation in the start of the yearly silver eel run. Low mean water temperatures during July–August and high mean water discharge during August–October resulted in an early start of the yearly silver eel run, whereas high mean water temperature and low water discharge gave the opposite effect. There was no significant correlation between the duration of the entire yearly silver eel run and the environmental variables investigated. But the duration of a part of the run, e.g. number of days from 5 to 25% cumulative eel descent, was significantly correlated with mean water temperature and increase in water discharge for the period in question ($R^2 = 0.84$). Maximum silver eel descent was at a water temperature of 9°C. Few silver eels descended at temperatures below 4°C or above 18°C. The migration speed of transplanted silver eels in the River Imsa was correlated with water discharge and water temperature ($R^2 = 0.88$). Water discharge alone explained 85% of the total variation in migration speeds. The recapture rate of silver eels transplanted within the River Imsa was highest at 9°C and decreased at higher and lower temperatures ($R^2 = 0.95$). Illumination of 20 lx upon the river reduced the descent of silver eels.

Nous avons examiné le lien entre la dévalaison des anguilles argentées d'Europe (*Anguilla anguilla*) et le débit de l'eau, sa température, la turbidité, la photopériode, l'éclairement et les phases de la lune, à partir de données et d'expériences sur le terrain couvrant une période de 10 ans. La migration des anguilles se produit entre août et décembre, et la température moyenne de l'eau entre juillet et août et le débit moyen de l'eau pendant la même période expliquaient 91 % de la variation totale du début de la dévalaison. Lorsque les températures moyennes étaient faibles en juillet–août, et le débit moyen élevé entre août et octobre, la dévalaison commençait plus tôt, alors que des températures élevées et un faible débit avaient l'effet contraire. Il n'y avait pas de corrélation significative entre la durée de l'ensemble de la dévalaison et les variables environnementales étudiées, mais la durée d'une partie de la dévalaison, par exemple le nombre de jours entre la dévalaison de 5 à 25 % du nombre cumulatif d'anguilles, présentaient une corrélation significative avec la température moyenne de l'eau et l'augmentation du débit pendant la période en question ($R^2 = 0,84$). La dévalaison des anguilles argentées était au maximum à une température de l'eau de 9°C, et les anguilles étaient rares à des températures inférieures à 4°C ou supérieures à 18°C. La vitesse de migration des anguilles argentées transplantées dans la rivière Imsa était corrélée avec le débit et la température de l'eau ($R^2 = 0,88$). Le débit de l'eau à lui seul expliquait 85 % de la variation totale dans les vitesses de migration. Le taux de recapture des anguilles argentées transplantées dans la rivière Imsa était au maximum à 9°C et diminuait lorsque la température était supérieure ou inférieure ($R^2 = 0,95$). Un éclairement de 20 lx sur la rivière réduisait la dévalaison.

Received October 25, 1985
Accepted June 25, 1986
(J8522)

Reçu le 25 octobre 1985
Accepté le 25 juin 1986

[1]Present address: Østfold County, Environmental Administration, P.O. Box 325, N-1501 Moss, Norway.

Each autumn, European silver eels (*Anguilla anguilla*) begin their migration from feeding areas in freshwater to the spawning area in the Sargasso Sea (Tesch 1977). The majority of the silver eels start their seaward migration shortly after their metamorphosis from yellow eels. Changes in photoperiod and temperature are probably "Zeitgebers" for the start of the metamorphosis (Hoar 1976; Purvis 1980; Clarke et al. 1981). It has been suggested that water flow, water temperature, and light intensity are environmental variables which regulate the migratory activity of silver eels (Lowe 1952; Deelder 1954, 1984; Boëtius 1967; Hain 1975; van Veen et al. 1976; Westin and Nyman 1979; Todd 1981; Haraldstad et al. 1985), but no experiment or statistical analysis has demonstrated the connection between these variables and migratory activity. The influence of the moon's phase on silver eel activity has been demonstrated experimentally, and this has been claimed to be an innate rhythm independent of light intensity (Korringa 1947; Jens 1952–53; Boëtius 1967; Hain 1975). However, the importance of the lunar cycle itself in regulating silver eel migration under natural conditions has also been questioned (Lowe 1952; Sloan 1984; Haraldstad et al. 1985).

Silver eels migrate during the night. Absence of day activity is probably a light avoidance behaviour; eels burrow under stones and in the mud during periods of strong light intensity (Lowe 1952; van Veen et al. 1976; Todd 1981; Sloane 1984; Haraldstad et al. 1985), perhaps to avoid predators.

The purpose of this study was to identify the key environmental variables influencing the downstream migration of silver eels. We examined the relationship between the descent of silver eels and water discharge, water temperature, photoperiod, moon phase, nocturnal light intensity, and water turbidity. This was done by (1) analysing field data on European silver eel downstream migration in the River Imsa, 1975–84, (2) measuring migration speed and recapture rates of transplanted silver eels in the River Imsa under naturally varying environmental conditions, and (3) comparing the intensity of silver eel migration from the River Imsa for periods with and without illumination at a fish trap.

Methods

The study was carried out in the River Imsa near Stavanger, southwestern Norway, 1975–84 (Haraldstad et al. 1985, their fig. 1). The catchment area is 128 km^2, of which 12% is lake surface. The water discharge varied between 0.5 and 30 $m^3 \cdot s^{-1}$. Mean yearly discharge was 4.5 $m^3 \cdot s^{-3}$. Mean current velocity in the river at yearly mean water discharge was approximately 0.75 $m \cdot s^{-1}$ (Hansen and Jonsson 1985).

Eels ascended up to 25 km up the river (Haraldstad et al. 1985). The numbers of descending silver eels captured at the river mouth using a Wolf trap (Wolf 1951) were recorded daily together with water discharge and water temperature at 08:00 from 1975 to 1984.

The transplantation experiments were performed in autumn 1984. Descending silver eels captured in the fish trap were anaesthetized with buffered tricaine methanosulphonate (MS-222 Sandoz) and tagged with Floy tags (Table 1). The silver eels were tagged within 14 d of capture in the trap. After tagging, they were allowed to recover for 24 h and were then released in batches of from 60 to 100 into the River Imsa 1000 m above the trap at the outlet of the lowest lake in the watercourse, Lake Liavatnet. The current velocity at the site of release was approximately 1 $m \cdot s^{-1}$. We recorded the time from release to recapture in the trap. Water temperature and water turbidity (absorbance, optical density at 720 nm, Shimadzu UV-210A spectrophotometer) were recorded daily at 08:00. We also recorded light intensity (lux) between 20:00 and 22:00 (United Detector Technology, 81 optometer). Data on photoperiod and moon phase were obtained from the Institute of Astrophysics, University of Oslo.

When testing possible correlations between the silver eel descent and subsets of the environmental variables, multiple regression analysis was used. The SAS computer program ('The *R* square Procedure," Ray 1982) tested for all possible regressions between cumulative eel descent (Y) (dependent variable) and water discharge (X_d), water temperature (X_t), and moon phase (X_m) (independent predictor variables) (model: $Y = A_1 + A_2X_d + A_3X_t + A_4X_m$). The regressions were performed on both untransformed and $\log_e$-transformed data, and the model giving the better fit was chosen. Models having partial regression coefficients with $P > 0.10$ were rejected (Sokal and Rohlf 1981).

When analysing the field data for the start of the yearly migration of eels, water temperature was expressed as the mean for July and August and water discharge was expressed as the mean for the period 1 August – 31 October. The migration started within the latter 3 mo each year. When analysing the duration of the run, mean water temperature and the increase in water discharge (fitted by the least squares method) for the periods in question were used. A number of other ways of presenting water discharge and temperature were tested, but they did not alter our finding. Regression analyses for the daily rate of eel descent and the measured environmental variables were also made, but no significant results were obtained.

In the transplantation experiments, we tested the regression between the dependent variables migration speed and rate of recapture and the independent variables water discharge, water temperature, water turbidity, light intensity, photoperiod, and moon phase. Migration speed is given as number of days from release to 50% of total cumulative recapture in the trap. The independent variables are given as means for the period from release to 50% of total cumulative recapture. The recapture rates are given as the ratio between total recapture during 1984 and number released of each batch. The independent variables in this analysis are given as means for the period from release to total recapture of the batch.

To test if light affected the migratory activity of the silver eels, two lamps at the weir on each side of the river (10 m apart, 4 m above the ground) were switched on and off every 30 min during night. When on, light intensity measured at the darkest point across the river was 20 lx. When off, light intensity was between 0.15 and 0.007 lx. We counted how many silver eels descended during each 30-min interval. The effects of this procedure were tested with a χ^2-test.

Results

Field Study

The number of descending silver eels (Table 1) and the start of the downstream migration (Fig. 1) varied considerably between years. Usually, the run started ($Y_{5\%}$, defined as number of days after 1 August of 5% cumulative silver eel descent) earlier in years with low than in years with high mean water temperature during July and August (X_t, degrees Celsius):

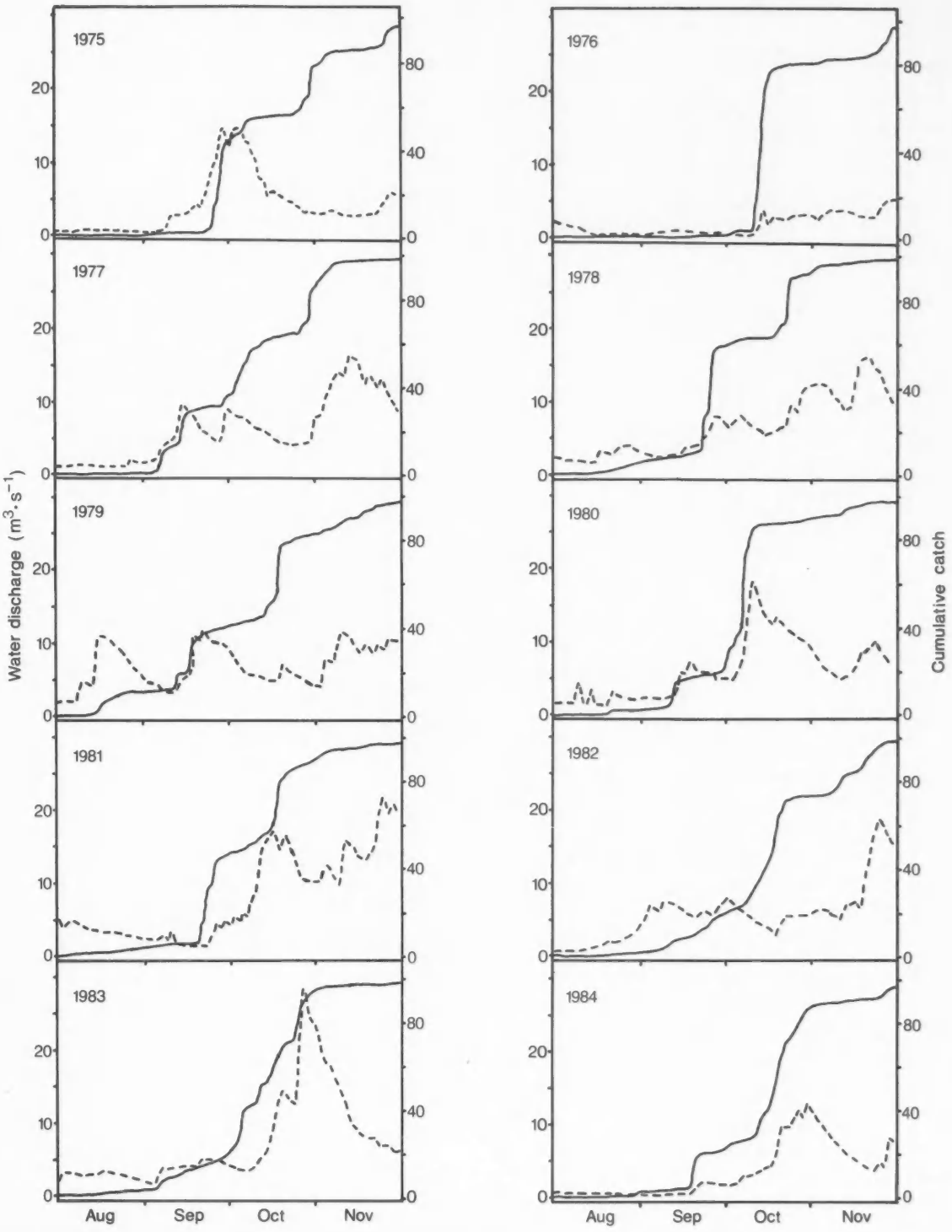


FIG. 1. Cumulative capture of descending silver eels in the fish trap (——) and water discharge (---) in the River Imsa during 1975–84.

TABLE 1. Number of silver eels, mean water temperature for July–August, water temperature (°C) at the day of 5% cumulative silver eel descent, mean water discharge between 1 August and 31 October ($m^3 \cdot s^{-1}$), and observed and predicted date (eq. 2) of 5% cumulative silver eel descent in the River Imsa.

Year	Number of eels	Water Temperature		Water discharge	Observed date	Predicted date
		July–August	5% descent			
1975	4836	19.7	12.3	4.366	26 September	30 September
1976	3813	19.0	10.8	1.600	12 October	5 October
1977	5257	17.4	13.9	4.135	7 September	10 September
1978	4525	16.6	14.8	4.801	27 August	31 August
1979	2626	15.4	15.0	6.393	17 August	15 August
1980	2996	17.4	14.3	5.916	10 September	3 September
1981	2354	16.5	15.6	5.873	22 August	27 August
1982	3323	18.0	13.1	4.430	11 September	14 September
1983	3595	17.1	14.8	6.067	6 September	31 August
1984	3169	17.4	12.9	2.563	17 September	18 September

TABLE 2. Date for 5 and 25% cumulative silver eel descent, mean water temperature (°C), and slope and intercept of water discharge ($m^3 \cdot s^{-1}$) on days, and observed and predicted number of days (eq. 3) between 5 and 25% cumulative silver eel descent in the River Imsa, 1975–84.

Year	Date for:		Mean water temperature	Water discharge		Number of days	
	5%	25%		Slope	Intercept	Observed	Predicted
1975	26 September	28 September	12.1	2.150	7.43	2	2
1976	12 October	13 October	10.6	0.600	0.60	1	6
1977	7 September	15 September	12.9	0.695	2.07	8	16
1978	27 August	24 September	13.2	0.042	2.59	28	24
1979	17 August	18 September	14.9	−0.191	9.80	32	35
1980	10 September	2 October	13.2	0.067	4.46	22	24
1981	22 August	23 September	15.0	−0.054	3.26	32	33
1982	11 September	9 October	12.4	0.002	6.48	28	21
1983	6 September	4 October	12.9	0.029	3.87	28	23
1984	17 September	3 October	12.0	0.057	2.10	16	18

(1) $Y_{5\%} = -170.27 + 12.05X_t$

$R^2 = 0.81$, $F_{1,8} = 33.27$, $P = 0.0004$, $P_{X_t} = 0.0004$.

Furthermore, the run started earlier in years with high water discharge during autumn. Using mean water discharge for August–October (X_d, metres per second) as a second independent variable, 91% of the total variation in $Y_{5\%}$ could be explained:

(2) $Y_{5\%} = -92.13 + 8.72X_t - 4.33X_d$

$R^2 = 0.91$, $F_{2,7} = 36.29$, $P = 0.0002$, $P_{X_t} = 0.00025$, $P_{X_d} = 0.023$.

Mean water discharge during August–October alone explained 65% of the total variation in start of the run ($Y_{5\%}$).

There was no significant correlation between moon phase and the start of the silver eel descent.

The duration of the yearly silver eel run varied between years. However, we found no significant relationship between the duration of the entire runs and the environmental variables investigated. There were, however, significant correlations between the duration of a part of the run and mean water temperature ($\bar{X}_t$, degrees Celsius and increase in water discharge (X_s, slope of the regression of water discharge on day) for the period in question. Using number of days from 5 to 25% of the yearly cumulative silver eel descent ($Y_{5-25\%}$) as the dependent variable, 84% of the variability in $Y_{5-25\%}$ could be explained (Table 2):

(3) $Y_{5-25\%} = -38.22 + 4.74\bar{X}_t - 9.90X_s$

$R^2 = 0.84$, $F_{2,7} = 18.13$, $P = 0.0017$, $P_{X_t} = 0.018$, $P_{X_s} = 0.012$.

The partial regression coefficient of the intercept of the fitted water discharge equations (Table 2) was not significant ($P = 0.27$), and this term was not added to eq. (3). Mean water temperature ($\bar{X}_t$) and increase in water discharge alone explained 57 and 62%, respectively, of the total variation in $Y_{5-25\%}$.

Thus, low water temperatures and high increases in water discharge increased the rate of descent early in the season. The reason why this regression does not hold true for the whole period of migration may be that (1) the water discharge fluctuated very much during the migration period making simple regressions, like eq. (3), meaningless, (2) there may be an optimal water temperature for leaving the river, and the rate of descent may decrease at both higher and lower temperatures, (3) the river contains a number of lakes with low water throughput, and silver eels from the upper parts of the river may be delayed in the lakes, (4) environmental conditions in the upper parts of the river may differ from those at the outlet, and all eels may not receive the same environmental stimuli at the same time, and (5) when those eels that will migrate have done so, the descent stops even when environmental conditions are

TABLE 3. Date of release, number released (N), and recaptured (R), mean water discharge ($m^3 \cdot s^{-1}$), mean water temperature (°C), mean turbidity (optical density $\times 10^{-4}$), and mean photoperiod (h) between date of release and date of 50% of total recapture, and observed and predicted number of days (eq. 5) between release and 50% of total recapture for transplanted silver eels in the River Imsa, 1984.

Date	N	R	Water discharge	Water temperature	Turbidity	Photoperiod	Observed	Predicted
9 September	99	86	1.87	11.75	24.57	11.79	30	25
21 September	99	84	2.29	11.29	26.86	11.29	21	18
26 September	97	90	2.49	11.05	19.44	11.08	18	16
5 October	100	90	3.28	10.44	17.80	10.62	10	10
8 October	98	88	3.60	10.20	17.75	10.44	8	9
12 October	98	95	4.33	9.99	8.29	10.14	7	7
17 October	99	96	4.95	9.80	3.00	9.91	2	6
24 October	99	91	10.90	9.15	19.50	9.29	2	1
26 October	99	93	12.20	8.80	53.00	9.09	1	2
31 October	100	81	12.20	9.10	26.00	8.44	1	1
8 November	94	78	6.60	7.94	19.50	7.75	8	4
22 November	60	41	4.83	5.28	22.00	6.83	6	8
11 December	100	60	6.48	5.62	13.80	6.25	5	5
21 December	99	45	9.30	5.50	4.67	6.00	2	3

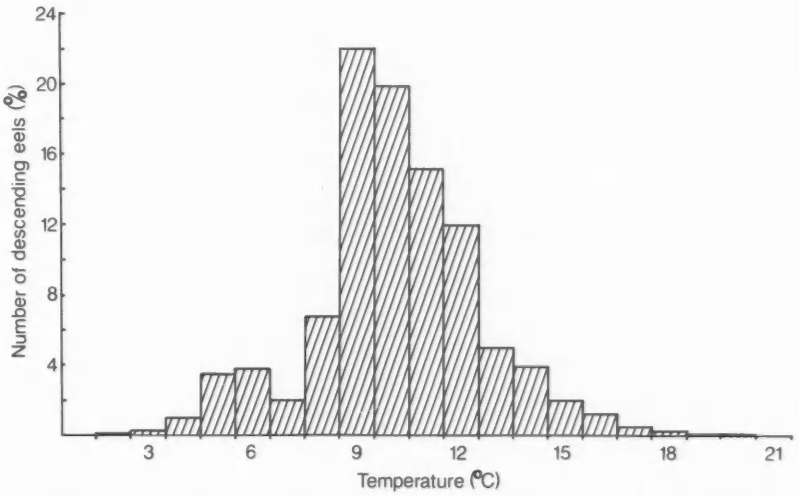


FIG. 2. Percent silver eel descent (N = 36 494) at temperatures between 2 and 20°C in the River Imsa during 1975–84.

favourable.

Checking for the second point, we found that most silver eels descended at temperatures between 9 and 12°C, with a maximum at 9°C (Fig. 2). Few silver eels descended at temperatures higher than 18°C or lower than 4°C. But, although water temperature significantly influenced the start of the run (eq. (1) and (2)), there was no threshold temperature at which the run started (Table 1).

To avoid the problem raised in points 1–5 above, a series of transplantation experiments were performed in the lower 1 km of the river, below the lowermost lake.

Transplantation Experiments

In the transplantation experiments, we observed large variations in the time between the release of the silver eels and their recapture in the fish trap (Table 3). In September, there was often a considerable delay between release and recapture. In October–December, however, most recaptures were made within a few days of release. The recapture rate within the same autumn was high in September and October (>80%), but decreased in November and especially in December. There were significant correlations between the number of days from release to 50% of total recapture ($M_{50\%}$) and mean water discharge (X_d) during the interval (eq. 4):

$$(4)\quad \log_e M_{50\%} = 4.25 - 1.56 \log_e X_d$$

$$R^2 = 0.85,\ F_{1,13} = 70.18,\ P < 0.0001,\ P_{X_d} < 0.0001.$$

Adding mean temperature (X_t) to the model increased the coefficient of determination significantly:

$$(5)\quad \log_e M_{50\%} = 6.04 - 1.75 \log_e X_d - 0.70 \log_e X_t$$

$$R^2 = 0.88,\ F_{2,12} = 43.12,\ P < 0.0001,\ P_{X_d} < 0.0001,\ P_{X_t} = 0.092.$$

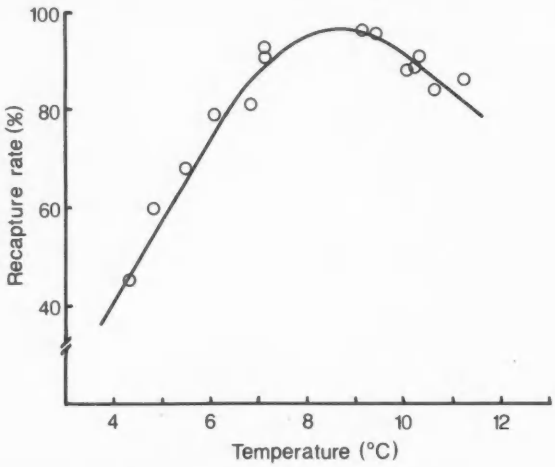


FIG. 3. Percent recapture of silver eels released in the River Imsa. Temperatures are means for the periods from release to last recapture. The curve is filled to the data by least squares regression (eq. 6).

Adding mean turbidity (X_o) to the model increased the coefficient of determination to $R^2 = 0.90$. However, turbidity may not be used as a predictor variable, as the partial regression coefficient of the term was not significant, $P_{X_o} = 0.17$. No other environmental variable (except photoperiod) contributed significantly to the best regression model found. Mean photoperiod (X_p) contributed almost equally as much to the model as did mean water temperature. The partial regression coefficient of this term was, however, not significant ($P_{X_p} = 0.115$). Furthermore, water temperature and photoperiod are highly correlated (e.g. in 1984: $R^2 = 0.94$), so only one of these variables can be used in the model.

The recapture rate of silver eels in the fish trap (N = number recaptured per number released) differed between the various batches released (release dates are given in Table 2) and was correlated with mean water temperature from the time of release to last recapture of each group (X_t). The best model found explained 95% of the total variation in N:

$$(6)\quad N = 1/(12.19 - 10.39 \log_e X_t + 2.42 (\log_e X_t)^2)$$

$$R^2 = 0.95,\ F_{2,11} = 109.3,\ P < 0.0001,\ P_{X_t} < 0.0001,$$

$$P_{X_t^2} < 0.0001.$$

The highest recapture rate was recorded at a mean water temperature of 9°C, and it decreased at both lower and higher temperatures (Fig. 3), as described in eq. (6). The eel migration stopped at low temperatures. Most of the tagged silver eels which did not descend during the autumn 1984 descended during autumn 1985.

Using mean photoperiod instead of water temperature in a corresponding regression model, we explained 93% of the variation in the recapture rate. As water temperature gives the better prediction, photoperiod was omitted. We found no significant correlation between recapture rate and any of the other environmental variables tested.

Illumination

Light at the weir reduced the descent of silver eels. During 29 half-hour periods of illumination (20 lx), 81 silver eels were caught. During the intervening dark periods, 362 silver eels were caught ($\chi^2 = 53.3$, 10 df, $P < 0.0001$). Most silver eels descended during the first dark period after sunset when light intensity was less than 0.06 lx. Within the dark periods, there was no significant regression between light intensity and number of descending silver eels, but the descent decreased when the light intensity was above approximately 0.06 lx.

Discussion

Water Temperature

Water temperature regulates the activity of poikilotherm animals including fish. In salmonids, water temperature influences the transformation from stream-dwelling parr to seaward migrating smolts (Clarke et al. 1981) as well as the timing of the sea run (Foerster 1937; Jonsson and Ruud-Hansen 1985), and it is likely that it has similar effects on eels. It may control the timing of the transformation from freshwater living yellow eels to seaward migrating silver eels and the start of their descent. In the River Imsa, silver eels started to migrate earlier in years with low than years with high summer temperatures. The eel descent started at quite different temperatures in different years, however, and there seemed to be no exact threshold temperature initiating the migration as suggested by Tesch (1977).

Both in nature and in our experiment, the rate of descent of silver eels was at its maximum at 9°C but decreased at both higher and lower temperatures. Few silver eels descended at temperatures above 18°C or below 4°C. Our tagging experiments in the River Imsa showed that silver eels that are stopped by low temperatures in any year descend during the following autumn. Thus, low temperatures decrease the activity of silver eels, as was pointed out by Tesch (1977). Westin and Nyman (1979) proposed that the activity of silver eels increases with decreasing water temperature, except for temperatures close to 0°C. Our results do not support this, and we find Westin and Nyman's (1979) results difficult to interpret, as no statistical justification of their contention is presented.

Thus, water temperature seems to be important for the start of the migration of silver eels and for the rate of their descent. It was, however, less important for the migration speed within the river.

Water Discharge

Flow of water in rivers has a controlling influence on the orientation of fish and on the intensity of their movements (Arnold 1974; Thorpe et al. 1981). The initiation and peaking of downstream movements of juvenile salmonids are, for instance, associated with freshets (Northcote 1984 and references therein). In the present study, high water discharge increased migration speed. Increasing migration speed may also be the reason why the rate of eel descent increased with increasing water discharge in the field study. However, each autumn there were periods with high or increasing water discharge when the rate of descent was low. Because of this, we could not predict the daily descent based on the environmental variables investigated. There is probably only a limited number of silver eels situated in the lower part of the river which are ready to leave the river at any given time. And, when they have descended, there may be no further migrants present. The great importance of water flow for silver eel descent is supported by a number of studies by other authors (e.g. Lowe 1952; Jens

1952–53; Deelder 1954, 1984; Hain 1975, Todd 1981).

The fact that the migration speed increased with increasing water discharge suggests that the silver eels are transported by the current. Similarly, Arnold and Cook (1984) suggested that silver eels use selective tidal stream transport when migrating in coastal areas. However, the silver eel river migration is probably a modulated displacement. They migrate downstream head first (Lowe 1952; Hain 1975) and must swim actively out of sloughs and backwaters and into the current. The downstream movement, however, is much slower than the current velocity in the River Imsa (Hansen and Jonsson 1985), and they seem to migrate only short distances each night. Transplanted silver eels took from 4 h to more than 1 mo to move 1 km downstream.

Thus, water discharge seems to be the key environmental variable determining the migration speed in rivers of European silver eels. This finding parallels those on several other fish species (Northcote 1984) and may have wide application in fish migration.

Photoperiod and Light

Photoperiod during spring is one of the factors controlling smolting of salmon (Wedemeyer et al. 1980), and it may also be important for the transformation from yellow to silver eels, as well as for the start of the migration of eels in the autumn. Moreover, for several fish species it is evident that seasonal changes in the start and cessation of downstream movement are controlled by shortening or lengthening of the day (Northcote 1962; Shershev and Zhulkov 1979). Advanced photoperiod schedules have advanced seasonal timing of downstream movement of steelhead (*Salmo gairdneri*) smolts by at least a month (Zaugg 1981). However, in the River Imsa we found no significant regression between photoperiod and silver eel descent. Photoperiods are the same on corresponding dates in different years; thus, this variable cannot be used as a predictor for yearly variation in silver eel descent. Furthermore the transplantation experiments indicated that photoperiod was less important than temperature for the rate of eel descent. But, there was high intercorrelation between water temperature and photoperiod during autumn. Thus the two variables cannot be distinguished properly using our models.

The silver eels usually started to migrate shortly after sunset and stopped when the moon appeared above the horizon and shined into the river. Furthermore, the silver eel descent was significantly reduced by illumination of 20 lx on the river. However, we found no significant regression between silver eel descent and light intensity during dark hours. Thus, this study indicates that there is a threshold intensity of light which inhibits the silver eel migration. This threshold may be at a level of approximately 0.06 lx at the small waterfall by the weir.

Except that few eels descended when the moon was above the horizon, we found no significant correlation between moon phase and silver eel descent in the River Imsa. This finding is in agreement with Lowe (1952) and Sloane (1984). Other authors have shown that silver eels held in tanks in complete darkness with constant temperature and water flow had an innate activity rhythm correlated with the lunar phase (Boëtius 1967; Edel 1976). It has been suggested that this innate activity rhythm also exists among silver eels in nature (Korringa 1947; Jens 1952–53; Hain 1975; Tesch 1977; Todd 1981; Deelder 1984).

As described above, we believe that the activity of silver eels is influenced by variations in light intensity. The innate lunar-correlated activity rhythm, on the other hand, may only be meaningful when eels are deprived of other sensory inputs. Around the full moon, migration may occur when the moon is below the horizon when other conditions (e.g. water temperatures and water discharge) are favourable (Haraldstad et al. 1985).

Acknowledgements

We are indebted to the manager Jon G. Backer and the staff at the Research Station for Freshwater Fish at Ims for helping us with daily sampling of eels throughout 10 yr. K. Hindar, R. H. K. Mann, A. Youngson, and P. S. Enger and two anonymous referee's have commented critically on drafts of the paper. M. Helgesen, G. Herjuaune, and N. Jonsson gave secretarial assistance. This research was supported by the Directorate for Nature Management and the Norwegian Fisheries Research Council.

References

ARNOLD, G. P. 1974. Rheotropism in fishes. Biol. Rev. Cambridge Philos. Soc. 49: 515–576.

ARNOLD, G. P., AND P. H. COOK. 1984. Fish migration by selective tidal stream transport: first results with a computer simulation model for the European continental shelf, p. 227–261. *In* J. D. McCleave, G. P. Arnold, J. J. Dodson, and W. H. Neill [ed.] Mechanisms of migration in fishes. Plenum Publishing Corporation, New York, NY.

BOËTIUS, J. 1967. Experimental indication of lunar activity in European silver eels, *Anguilla anguilla* (L.). Medd. Dan Fisk. Havunders. Ny Ser. 6: 1–6.

CLARKE, W. C., J. E. SHELBOURN, AND J. R. BRETT. 1981. Effects of artificial photoperiod cycles, temperature, and salinity on growth and smolting in underyearling coho (*Oncorhynchus kisutch*), chinook (*O. tshawytscha*), and sockeye (*O. nerka*) salmon. Aquaculture 22: 105–116.

DEELDER, C. L. 1954. Factors affecting the migration of the silver eel in dutch inland waters. J. Cons. Int. Explor. Mer 20: 177–185.

1984. Synopsis of biological data on the eel *Anguilla anguilla* (Linneaeus, 1958). FAO Fish. Synop. No. 80, Revision 1: 1–73.

EDEL, R. K. 1976. Activity rhythms of maturing American eels (*Anguilla rostrata*). Mar. Biol. 36: 283–289.

FOERSTER, R. E. 1937. The relationships of temperature to the seaward migration of young sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Board Can. 3: 421–438.

HAIN, J. H. W. 1975. The behaviour of migratory eels, *Anguilla rostrata*, in response to current, salinity and lunar period. Helgol. Wiss. Meeresunters. 27: 211–233.

HANSEN, L. P. AND B. JONSSON. 1985. Downstream migration of hatchery-reared smolts of Atlantic salmon (*Salmo salar* L.) in the River Imsa, Norway. Aquaculture 45: 237–248.

HARALDSTAD, Ø., L. A. VØLLESTAD, AND B. JONSSON. 1985. Descent of European silver eels, *Anguilla anguilla* L., in a Norwegian watercourse. J. Fish Biol. 26: 37–41.

HOAR, W. S. 1976. Smolt transformation: evolution, behavior, and physiology. J. Fish. Res. Board Can. 33: 1233–1252.

JENS, G. 1952–53. Über den lunaren Rhythmus der Blankaalwanderung. Arch. Fischereiwiss. 4: 94–110.

JONSSON, B., AND J. RUUD-HANSEN. 1985. Water temperature as the primary influence on timing of seaward migrations of Atlantic salmon (*Salmo salar*) smolts. Can. J. Fish. Aquat. Sci. 42: 593–595.

KORRINGA, P. 1947. Relations between the moon and the periodicity in the breeding of marine animals. Ecol. Monogr. 17: 347–381.

LOWE, R. H. 1952. The influence of light and other factors on the seaward migration of the silver eel (*Anguilla anguilla* L.). J. Anim. Ecol. 21: 275–309.

NORTHCOTE, T. G. 1962. Migratory behaviour of juvenile rainbow trout, *Salmo gairdneri*, in an outlet and inlet streams of Loon Lake, British Columbia. J. Fish. Res. Board Can. 19: 201–270.

1984. Mechanisms of fish migration in rivers, p. 317–355. *In* J. D. McCleave, G. P. Arnold, J. J. Dodson, and W. H. Neill [ed.] Mechanisms of migration in fishes. Plenum Publishing Co., New York, NY.

PURVIS, H. A. 1980. Effects of temperature on metamorphosis and the age and length of metamorphosis in sea lamprey (*Petromyzon marinus*) in the

Great Lakes. Can. J. Fish. Aquat. Sci. 37: 1827–1834.

RAY, A. A. 1982. SAS users guide: statistics. SAS Institute, North Carolina. 584 p.

SHERSHEV, A. P., AND A. I. ZHULKOV. 1979. Features of the downstream migration of young pink salmon and some indices of the efficiency of reproduction of the pink salmon, *Oncorhynchus gorbuscha*, from Pritorhaya River. J. Ichthyol. 19: 114–119.

SLOANE, R. D. 1984. Preliminary observations of migrating adult freshwater eels (*Anguilla australis australis* Richardson) in Tasmania. Aust. J. Mar. Freshwater Res. 35: 471–476.

SOKAL, R. R., AND F. J. ROHLF. 1981. Biometry. 2nd ed. W. H. Freeman and Company, San Francisco, CA.

TESCH, F.-W. 1977. The eel. Chapman and Hall, London. 434 p.

THORPE, J. E., L. G. ROSS, G. STRUTHERS, AND W. WATTS. 1981. Tracking Atlantic salmon smolts, *Salmo salar* L. through Loch Voil, Scotland. J. Fish Biol. 19: 519–537.

TODD, P. R. 1981. Timing and periodicity of migrating New Zealand freshwater eels (*Anguilla* spp.). N.Z. J. Mar. Freshwater Res. 15: 225–235.

VAN VEEN, T., H. G. HARTWIG, AND K. MÜLLER. 1976. Light-dependent motor activity and photonegative behaviour in the eel (*Anugilla anguilla* L.). J. Comp. Physiol. 111: 209–219.

WEDEMEYER, G. A., R. L. SAUNDERS, AND W. C. CLARKE. 1980. Environmental factors affecting smoltification and early marine survival of anadromous salmonids. Mar. Fish. Rev. 42(6): 1–14.

WESTIN, L., AND L. NYMAN. 1979. Activity, orientation, and migration of Baltic eel (*Anguilla anguilla* L.). Rapp. P.-V. Réun. Cons. Int. Explor. Mer 174: 115–123.

WOLF, P. 1951. A trap for the capture of fish and other organisms moving downstream. Trans. Am. Fish. Soc. 80: 41–45.

ZAUGG, W. S. 1981. Advanced photoperiod and water temperature effects on gill Na^+-K^+ adenosine triphosphatase activity and migration of juvenile steelhead (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 38: 758–764.

Impact of Atrazine on Periphyton in Freshwater Enclosures and Some Ecological Consequences

David Herman and Narinder K. Kaushik

Department of Environmental Biology, University of Guelph, Guelph, Ont. N1G 2W1

and Keith R. Solomon

Canadian Centre for Toxicology, 645 Gordon Street, Guelph, Ont. N1G 2W1

Herman, D., N. K. Kaushik, and K. R. Solomon. 1986. Impact of atrazine on periphyton in freshwater enclosures and some ecological consequences. Can. J. Fish. Aquat. Sci. 43: 1917–1925.

The herbicide atrazine inhibited production of periphyton within limnocorrals (in situ aquatic enclosures) and altered the composition of the periphyton community. On two occasions, 6 wk apart, three limnocorrals were treated with a surface application of atrazine equivalent to a nominal enclosure concentration of 100 $\mu g \cdot L^{-1}$. During the first atrazine pulse, the Cyanophyta disappeared and organic matter decreased to values significantly less than those within three control enclosures. Within 3 wk of the second atrazine application, a 36–67% reduction in organic matter, chlorophyll, and algal biomass and a significant decrease in the rate of carbon assimilation were evident. These events coincided with a decrease in number of certain Chlorophyta species, although other Chlorophyta species increased in number. The Bacillariophyceae maintained biomass levels greater than those in the controls for 9 wk after the second atrazine application. Total limnocorral periphyton production was greater than that of the phytoplankton after 5–10 wk of periphyton colonization in both the control and atrazine limnocorrals.

L'atrazine, un herbicide, a inhibé la production du périphyton dans les limites de limnocorrals (enceintes établies dans le milieu aquatique) et en a modifié la composition. Par deux fois, à 6 sem d'intervalle, trois limnocorrals ont reçu en surface une application d'atrazine correspondant à une concentration de 100 $\mu g \cdot L^{-1}$. Après le premier traitement, les Cyanophytes sont disparus et la matière organique a diminué jusqu'à des valeurs sensiblement inférieures à celles observées dans les trois enceintes témoins. Dans les 3 sem suivant la deuxième application, on a constaté une diminution de 36 à 67 % de la matière organique, de la chlorophylle et de la biomasse des algues et une réduction sensible du taux d'assimilation du carbone. Ces événements ont coincidé avec une diminution du nombre de certains Chlorophytes, alors que d'autres ont augmenté. La biomasse des Bacillariophycés s'est maintenue à un niveau supérieur à celui des enceintes témoins pendant 9 sem après la deuxième application d'atrazine. Après 5 à 10 sem de colonisation des enceintes témoins et des limnocorrals par le périphyton, la production périphytonique totale des limnocorrals a été supérieure à la production phytoplanctonique.

Received January 8, 1986
Accepted June 17, 1986
(J8643)

Reçu le 8 janvier 1986
Accepté le 17 juin 1986

Repeated agricultural use of environmentally persistent herbicides has led to concern over the impact of such chemicals on the aquatic environment and more specifically on their toxicity to algae. In our study the herbicide atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine), which is used primarily for weed control in corn, was chosen as a test herbicide because of its extensive use (Canadian Centre for Toxicology 1984), high water solubility, low bioconcentration factor (Lynch et al. 1982), and persistence in soil with a half-life of more than 1 yr (Jones et al. 1982). Surface waters surrounded by agricultural land receive several doses of atrazine over the growing season because of surface runoff during rainstorms (Muir et al. 1978; Frank and Sirons 1979). Therefore, a pulsed atrazine treatment, as used in this study, closely represents real world conditions.

Laboratory studies on the toxicity of atrazine, an inhibitor of the electron transport chain (Moreland 1980), have shown that certain algal species are sensitive to concentrations as low as 1 $\mu g \cdot L^{-1}$ (Loeppky and Tweedy 1969; Butler et al. 1975; Torres and O'Flaherty 1976; Veber et al. 1981; Stratton 1984). Examination of the impact on phytoplankton in microcosms (Brockway et al. 1984) and in artificial ponds (DeNoyelles et al. 1982; Lay et al. 1984) has shown that 20 μg atrazine $\cdot L^{-1}$ can inhibit photosynthesis. Using field enclosures, Plumley and Davis (1980) found that a 2.2 $mg \cdot L^{-1}$ dose of atrazine had no consistent effect on salt marsh edaphic diatoms. Goldsborough and Robinson (1983) found that the EC50 of two herbicides closely related to atrazine, on the photosynthesis of freshwater marsh diatoms, was between 100 and 1000 $\mu g \cdot L^{-1}$ for simazine and less than 10 $\mu g \cdot L^{-1}$ for terbutryn.

The walls of in situ aquatic enclosures, or limnocorrals, provide a large surface area for the growth of attached organisms, primarily algae, which may contribute greatly to total primary production within the enclosures (Kemmerer 1968; Klussmann

and Inglis 1968; Lund 1972). We examined the attached organisms, commonly called periphyton, within freshwater limnocorrals because the impact of atrazine on periphyton of a freshwater lake has not been previously studied. The limnocorrals used were part of an experiment to evaluate the use of enclosures for pesticide impact studies (Kaushik et al. 1985; Solomon et al. 1985). Our objective was to determine the impact of atrazine on the periphyton community within the enclosures. We also compared the periphyton build-up within the enclosures with the phytoplankton biomass levels.

Materials and Methods

Construction and installation of the limnocorrals in Lake St. George, a 10.3-ha mesotrophic lake located in southern Ontario, have been described by Solomon et al. (1980, 1985). Briefly, the enclosures (5 × 5 × 5 m deep) were constructed using a single piece of yellow nylon-reinforced polyvinyl chloride (PVC). The material was supported on the water surface by floats and the sides of the limnocorrals were anchored firmly into the sediment, thus isolating a section of the water column and sediment layer. Three limnocorrals were used as controls and three were treated with atrazine.

The walls of the enclosures were lowered on May 5, 1983, and remained in place until July 1984. Periphyton colonization substrates were colonized for 3 wk before the first atrazine treatment. The limnocorrals received an atrazine application on June 1, 1983, and on July 6, 1983 (day 35). Atrazine (80% A.I.) was applied to the water surface, using a backpack spray system, to give a nominal enclosure concentration of 100 $\mu g \cdot L^{-1}$ with each application. Complete mixing of the atrazine in the enclosures occurred within 24 h. There were five sampling days (1, 5, 14, 24, and 34) in the first pulse and seven sampling days (37, 42, 54, 68, 96, 137, and 329) in the second pulse.

Atrazine residue analysis was carried out as described by Yoo and Solomon (1981). Due to the mechanical isolation of the enclosed water column and hence the elimination of inorganic nutrient renewal, the limnocorrals were supplemented with weekly nutrient additions at a rate equivalent to 6.5 g $N \cdot m^{-2} \cdot yr^{-1}$ (as sodium nitrate) and 0.5 g $P \cdot m^{-2} \cdot yr^{-1}$ (as phosphoric acid).

Periphyton Sampling

Periphyton colonization substrates were made of 6.5 × 4 cm strips of the same PVC material used for the limnocorrals; two strips were attached to a holder using silicone glue. To construct a holder, three microscope slides (2.5 × 10 cm) were attached along their short edge to a 20-cm length of glass tubing using silicone glue. A sampling unit was created by tying together, with nylon string, four such holders spaced at 0.5, 1.5, 2.5, and 3.5 m depths. Twelve sampling units were placed in each limnocorral, and on each sampling day one unit was randomly selected and brought to the surface. One strip from a holder at each depth was immediately preserved in Lugol's solution (American Public Health Association 1976) to be used for direct enumeration. Using a scalpel, periphyton from the second strip was scraped into distilled water and mixed until homogeneous in a Waring blender. Aliquots of the resulting suspension were analyzed for chlorophyll and organic matter content.

Organic Matter and Chlorophyll *a* Analysis

To determine organic matter, periphyton samples were filtered onto a preashed, preweighed Whatman GFC (2.4-cm diameter) filter and dried at 70°C for approximately 24 h. Each sample was weighed, ashed at 500°C for 1 h, and reweighed. The dry weight, less the ashed weight, gave the ash-free-dry-weight (AFDW), which was used as an estimate of organic matter.

Periphyton samples were filtered onto Whatman GFC (2.4-cm diameter) filters and frozen until chlorophyll extraction. Chlorophyll extraction followed the methods outlined by Marker et al. (1980) and Arvola (1981). Spectrophotometric determination (Bausch and Lomb Spectronic 88) of chlorophyll *a* was carried out using the procedure and equations given by Nusch (1980).

Direct Counts and Cell Biomass Calculations

Enumeration of the periphytic algae was carried out following the procedure outlined by Lund et al. (1958). The periphyton was scraped from the PVC strips into distilled water and mixed in a Waring blender. A subsample of the suspension was allowed to settle for a minimum of 12 h in a 50-mL phytoplankton sedimentation chamber. A minimum of 300 cells were counted per sample. The blending procedure fragmented many filamentous algae into short chains and in these, individual cells were counted. For the less easily fragmented Cyanophyta filaments, the length of the filament was measured and the number of cells calculated using an average cell size. Length, width, and depth measurements on a random selection of three to six cells of each species were used to calculate volume, assuming that the form of the algae corresponded to a simple geometric solid. Biomass was estimated from volume, assuming a cell density the same as water.

Periphyton and Phytoplankton Productivity Measurements

Photosynthetic activity of periphyton was measured in situ using ^{14}C assimilation as an estimate of net primary productivity. A microscope slide was attached on edge and perpendicular to the inside surface of a lid for a 250-mL glass incubation bottle using silicone glue. An injection port was drilled through the lid and sealed with silicone glue. A 6.5 × 2.5 cm strip of PVC was sealed to the slide, with silicone glue, for periphyton colonization; each lid was attached to a holder as described earlier. Four holders were tied together for periphyton sampling at 0.5-, 1.5-, 2.5-, and 3.5-m depths. Three sets of holders were suspended in each limnocorral on May 6 and allowed to colonize for 35 d before the first productivity measurement. Because of the time-consuming nature of the productivity measurements, only two limnocorrals, one control and one treated, were sampled each day. Three productivity sampling periods, two in the first pulse and one in the second, were carried out, with each sampling period lasting 3 d.

Using a Kemmerer sampler, water samples from the limnocorrals, at the above depths, were dispensed into separate 250-mL incubation bottles. Lids, with the attached periphyton sample, were fitted to the bottles. The bottles were suspended at the sampling depth for a 30-min temperature stabilization period before being brought to the surface, injected with 74 kBq of ^{14}C-labelled sodium bicarbonate (Amersham, Oakville, Ont.), and lowered and incubated for 2 h. All incubations were carried out in the same control limnocorral between 10:00 and

TABLE 1. Mean water chemical and physical values for the control limnocorrals (C) and the atrazine limnocorrals (A) for the first and second atrazine pulse periods in 1983 and for the April 1984 sampling day. *Significant difference between the means (ANOVA) at the 95% confidence level.

	Pulse 1 (n = 18)		Pulse 2 (n = 36)		April 1984 (n = 2)	
	C	A	C	A	C	A
Oxygen ($mg \cdot L^{-1}$), 0.5 m	9.26	9.45	9.90	7.80*	9.70	10.10
Oxygen ($mg \cdot L^{-1}$), 3.5 m	5.67	5.45	6.54	5.20*	9.70	10.10
Temperature (°C), 0.5 m	18.7	19.1	23.0	23.0	6.0	6.2
Temperature (°C), 3.5 m	10.5	10.0	18.6	18.3	6.0	6.2
Secchi depth (m)	3.2	3.5	3.3	4.4*	2.4	2.3
DIC ($mg \cdot L^{-1}$)	36.08	36.53	23.74	38.54*	29.9	31.3
NO_3-NO_2-N ($mg \cdot L^{-1}$)	0.317	0.250	0.235	0.477*	0.107	0.335
Total nitrogen ($mg \cdot L^{-1}$)	0.639	0.667	0.719	0.748	0.846	0.881
Total phosphorus ($mg \cdot L^{-1}$)	0.025	0.022	0.021	0.018	0.025	0.025

14:00.

After incubation, periphyton was scraped from the PVC strip and filtered onto a Millipore (45-μm pore size) filter. A drop of 0.5 *N* HCl was added to the filter to eliminate any labelled bicarbonate from the water retained by the filter paper (Lean and Burnison 1979). The filters were placed in a scintillation bottle containing 3 mL of 2-methoxyethanol and 12 mL of Aquasol-2 (New England Nuclear). Radioactivity was measured in a Packard Tri-Carb 460C liquid scintillation system using the sample channel ratio method to correct for quenching due to detritus or chlorophyll.

During one periphyton productivity measurement, phytoplankton samples from four depths were incubated in 500-mL bottles at the same time as the periphyton. The bottles were injected with 111 kBq of ^{14}C-labelled sodium bicarbonate through a port in the lid and incubated for 3 h. Phytoplankton ^{14}C assimilation was measured as described for the periphyton.

Light readings were taken using a Li-Cor spherical quantum sensor (Li-1935b) sensitive to photosynthetically active radiation (400–700 nm) and recorded on a Li-Cor (Li-510B) data integrator. Carbon assimilation was calculated according to Vollenweider (1969).

Water Chemistry and Physics

Weekly temperature and dissolved oxygen profiles were taken using a YSI model 51B (Yellow Springs Instrument Co.) dissolved oxygen meter. Weekly water samples were collected using a depth integrated sampler (Solomon et al. 1982) and analyzed for Kjeldahl nitrogen, combined nitrate nitrite nitrogen (NO_3-NO_2-N), total phosphorus, dissolved inorganic carbon (DIC), and phytoplankton chlorophyll *a* using standard methods (Environment Canada, 1979).

Statistical Analysis

The ANOVA procedure was used in the analysis of AFDW, chlorophyll, and carbon assimilation rates, with sampling depth considered as a separate factor. ANOVA was carried out for each sampling day (or measurement period) and the standard error was calculated using the pooled estimate of variance. For algal biomass, the means and standard errors were calculated for each sampling depth.

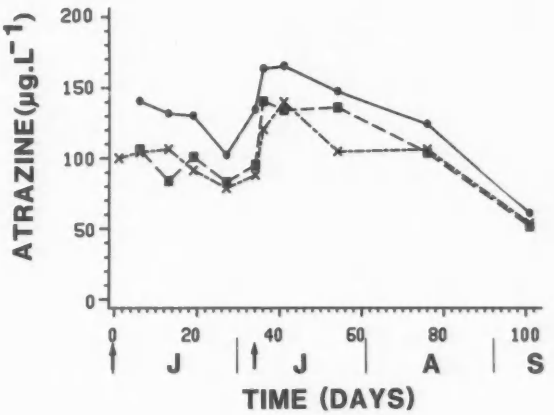


FIG. 1. Atrazine concentration in the water of the three treated limnocorrals in the first and second pulse. Arrows indicate day of atrazine application.

Results

Comparison of Control and Atrazine-Treated Limnocorrals

Atrazine concentrations ranged from 80 to 140 $\mu g \cdot L^{-1}$ during the first pulse (Fig. 1). The second atrazine application did not double the atrazine concentration but did increase the level by 20–30% in all limnocorrals.

There was no significant difference between atrazine and control limnocorrals for any chemical or physical parameter during the first pulse (Table 1). Throughout the second pulse (excluding day 329), oxygen concentrations at 0.5 and 3.5 m were significantly lower, while Secchi depth, DIC, and NO_3-NO_2-N were significantly greater in the atrazine limnocorrals.

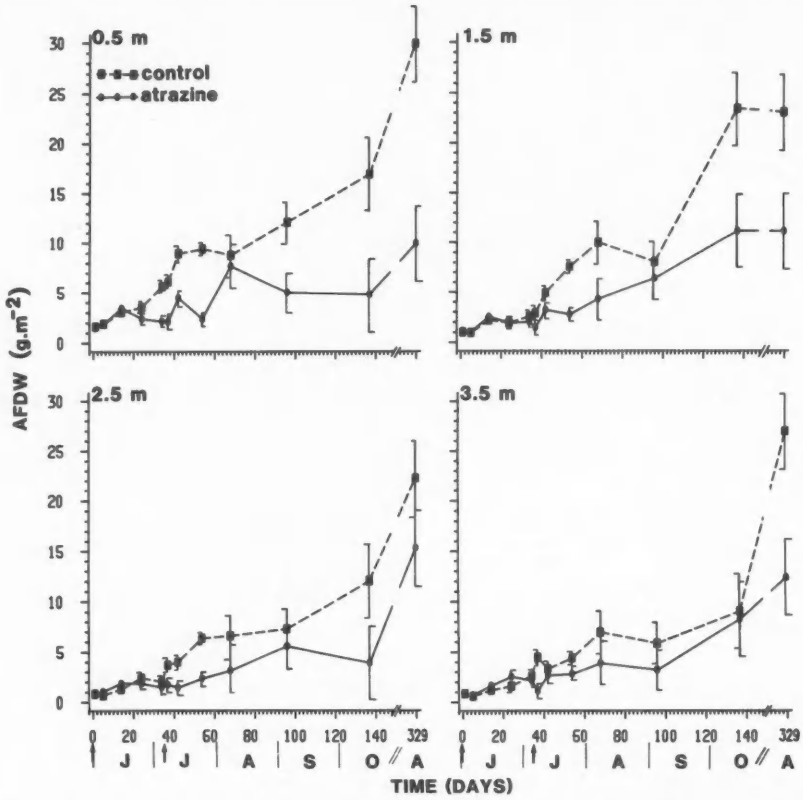


FIG. 2. Mean AFDW values for the periphyton in the control and atrazine-treated limnocorrals at four depths in the first and second pulse. Error bars represent one standard error and arrows indicate atrazine treatment.

By day 329, all parameters were similar in all limnocorrals, with the exception of the NO_3-NO_2-N, which remained at greater levels within the atrazine limnocorrals.

In the controls, AFDW values were greater than those of the atrazine limnocorrals after day 14 at most depths (Fig. 2). Significantly lower AFDW values in the treated limnocorrals were recorded at 0.5 m on day 34, the day before the second atrazine application, and at 0.5 and 1.5 m on most of the subsequent sampling days.

There were no significant differences in chlorophyll values between the control and atrazine treated limnocorrals (Fig. 3) until 19 d after the second atrazine application (day 54). For the remainder of the summer, chlorophyll levels within the atrazine limnocorrals were consistently lower than those of the controls, although not all differences were significant. Periphyton organic matter decreased with increased depth of sampling, although there was no effect of depth on total chlorophyll.

Algal groups present in the periphyton were the Cyanophyta (blue-green), Chlorophyta (green) and the Bacillariophyceae (diatom). On the first sampling day, the periphytic algae were dominated by the pennate Bacillariophyceae in most control and atrazine limnocorrals (Table 2). The most abundant species were *Fragilaria crotonensis* and several species of *Synedra*. Also common were *Achnanthes minutissima*, *Tabellaria fenestrata*, *Cymbella cistula*, *Gomphonema angustatum*, *G. truncatum*, and *Navicula radiosa*. Following the spring bloom the Bacillariophyceae, in both the control and atrazine limnocorrals, were dominated by *Achnanthes minutissima* and *Gomphonema angustatum*.

For the remainder of the summer, the Chlorophyta group dominated total algal biomass and total cell numbers in the control and atrazine limnocorrals. The Chlorophyta community in the control limnocorrals was dominated by the filamentous species, with *Oedogonium* sp. and *Bulbochaete* sp. contributing the greatest amount to total Chlorophyta biomass (Fig. 4). Within the atrazine limnocorrals, *Coleochaete* sp. was the dominant species. Dominant Cyanophyta species within the control limnocorrals were *Oscillatoria* sp. and unidentified cyanobacteria filaments with *Tolypothrix* sp. increasing in number by September.

The impact of atrazine on Cyanophyta was drastic. During the first pulse the Cyanophyta species almost completely disappeared from the atrazine limnocorrals (Fig. 5), although no impact on Chlorophyta and Bacillariophyceae was evident. After the second atrazine application, Chlorophyta and Bacillariophyceae biomass decreased to levels less than that in the controls on days 42 and 96, respectively, at most depths. The exception was the Chlorophyta at 3.5 m, where no difference in biomass levels was evident.

By the spring of 1984 (day 329), within the control limnocorrals, Bacillariophyceae and Cyanophyta had increased in their relative contribution to biomass and numbers. In the

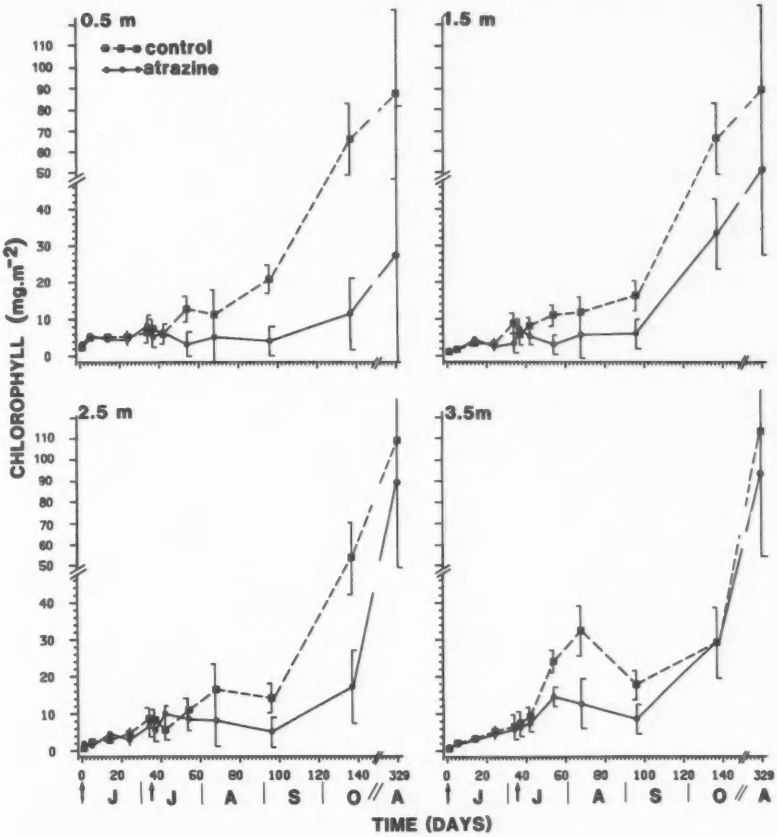


FIG. 3. Mean chlorophyll values for the periphyton in the control and atrazine limnocorrals at four depths in the first and second pulse. Error bars represent one standard error and arrows indicate atrazine treatment.

TABLE 2. Mean percentage total algal biomass and numbers for the periphytic Cyanophyta, Chlorophyta, and Bacillariophyceae groups on six sampling days. Percentages were averaged over the four sampling depths with the standard deviation (n = 12) shown in parentheses.

	Sampling day	% biomass			% numbers		
		Cyanophyta	Chlorophyta	Bacillariophyceae	Cyanophyta	Chlorophyta	Bacillariophyceae
				Control limnocorrals			
Pulse 1	1	1.3 (2.3)	21.1 (20.5)	77.7 (19.3)	30.6 (38.7)	22.1 (18.9)	47.3 (25.7)
	34	1.7 (2.6)	89.6 (11.4)	8.8 (11.7)	18.8 (19.7)	62.5 (26.2)	18.7 (19.5)
Pulse 2	42	1.9 (3.0)	90.8 (10.9)	7.3 (11.3)	25.1 (22.2)	56.8 (23.3)	18.2 (15.9)
	68	0.9 (1.4)	94.3 (6.9)	4.8 (5.9)	14.0 (24.8)	94.3 (24.2)	9.3 (6.9)
	96	4.8 (6.3)	88.8 (9.8)	6.3 (7.5)	28.5 (28.8)	55.8 (31.2)	15.7 (18.0)
	329	15.9 (22.4)	26.6 (3.8)	57.0 (35.5)	51.4 (16.8)	3.8 (4.5)	44.8 (18.2)
				Atrazine limnocorrals			
Pulse 1	1	2.5 (4.5)	24.8 (21.2)	72.7 (19.9)	24.4 (22.0)	17.5 (16.3)	58.1 (20.0)
	34	<0.1	88.8 (8.2)	11.2 (8.2)	0.8 (2.9)	68.0 (16.3)	31.2 (15.5)
Pulse 2	42	0	90.7 (5.2)	9.3 (5.2)	0	75.1 (14.2)	24.9 (14.2)
	68	0	93.2 (4.7)	6.8 (4.7)	0	60.0 (26.0)	40.0 (26.0)
	96	0	96.5 (3.0)	3.5 (3.0)	0	79.3 (18.8)	20.7 (18.8)
	329	0	62.4 (18.1)	37.6 (18.1)	0	25.7 (28.5)	74.3 (28.5)

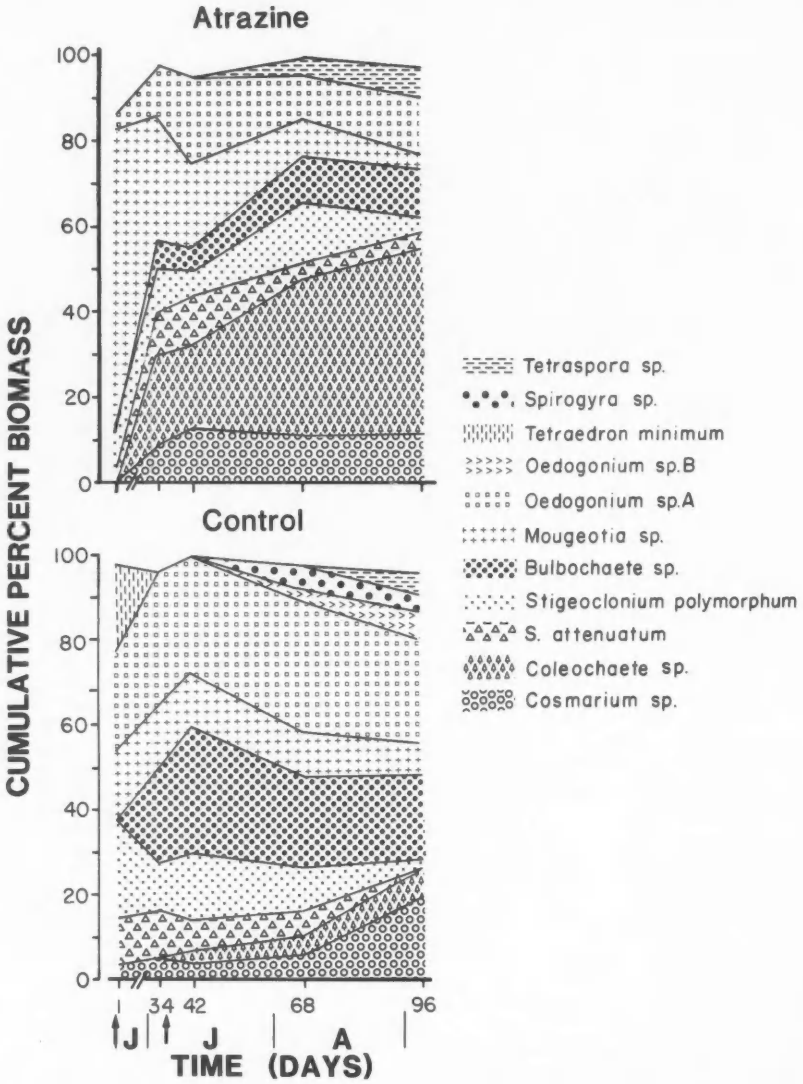


FIG. 4. Composition of the Chlorophyta community in the control and atrazine limnocorrals in the first and second pulse. Composition is described using the mean percent contribution of the common Chlorophyta species (averaged over the four depths) to total Chlorophyta biomass. Arrows indicate atrazine treatment.

atrazine limnocorrals, the Chlorophyta remained dominant in terms of biomass and the Bacillariophyceae dominated total cell numbers. Biomass in the atrazine limnocorrals showed no recovery to the levels evident in the controls.

Carbon assimilation measurements during the first pulse (Fig. 6) showed no significant difference in periphyton productivity between the control and atrazine limnocorrals. No carbon fixation measurements were possible for 0.5 m in the atrazine limnocorrals during the second pulse (days 47–49) because the PVC strips were lost. The ANOVA was performed for the remaining depths only. During the second pulse, significantly lower carbon assimilation rates occurred in the atrazine limnocorrals at 1.5 and 2.5 m.

Comparison of Periphyton and Phytoplankton Components of the Limnocorrals

Chlorophyll and productivity measurements were expressed on a per enclosure basis by multiplying the mean value of the four sampling depths by the total surface area of the walls for the periphyton and the nominal volume of the enclosure for the phytoplankton. Algae on the sediment surface were not sampled and therefore not included in the periphyton calculation.

Phytoplankton chlorophyll values were greatest in the early spring in both the control and atrazine limnocorrals (Fig. 7). Periphyton chlorophyll values increased with time to reach a maximum level by the end of the summer. In both the control

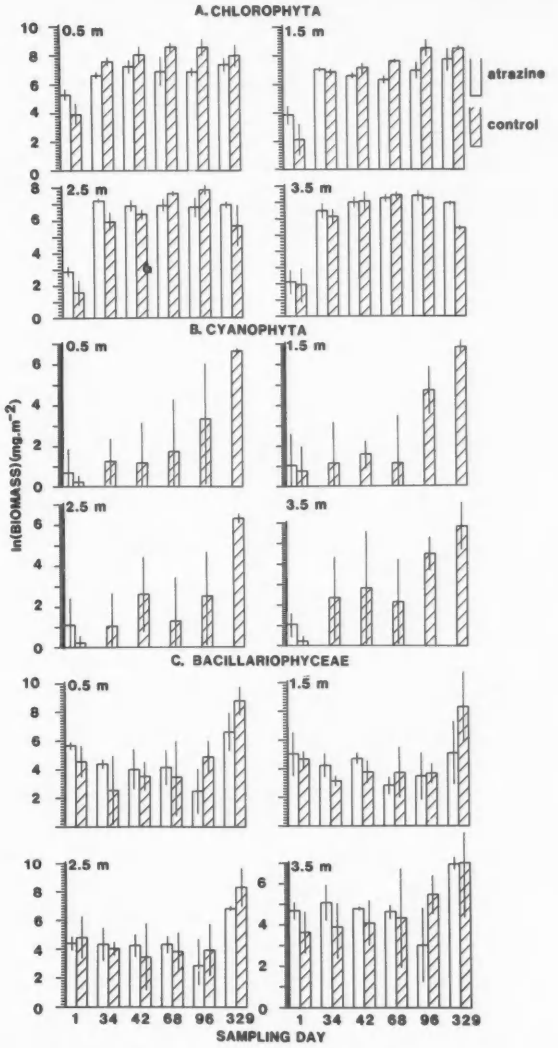


FIG. 5. Mean biomass values (natural log transformation) for the three periphytic algal groups in the control and atrazine limnocorrals at four depths on six sampling days. Error bars represent one standard error for the mean at each depth (n = 3). Atrazine treatment occurred on days 0 and 35.

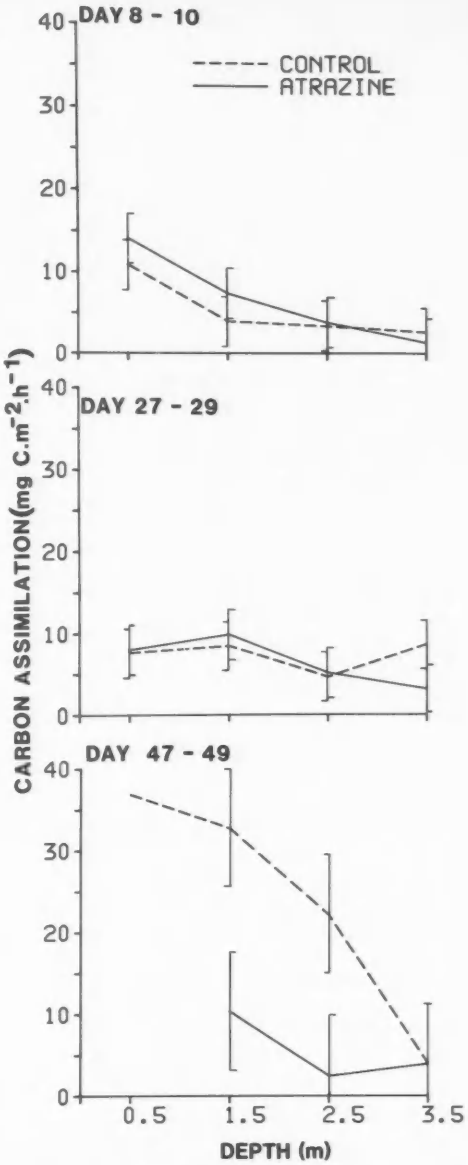


FIG. 6. Mean carbon assimilation rates for the control and atrazine limnocorrals for two measurement periods in the first pulse and one in the second pulse. Error bars represent one standard error. The second atrazine treatment was on day 35.

and atrazine limnocorrals, the periphyton chlorophyll values were greater than those of the phytoplankton after 4–5 wk of periphyton colonization.

No periphyton productivity measurements were made at 0.5 m in the atrazine limnocorrals because the PVC strips were lost. Total limnocorral productivity was calculated using the mean of the remaining depths. Simultaneous productivity measurements (Table 3) demonstrated that, after 10 wk of colonization, periphyton productivity could equal or exceed that of the phytoplankton.

Discussion

The results of our study suggest that the impact of atrazine on periphyton is dependent on the composition of the periphyton community at the time of exposure. During the first pulse, AFDW measurements and direct counts revealed a decrease in organic matter level and the sensitivity of the Cyanophyta to atrazine. Atrazine impact on the rate of carbon assimilation and total chlorophyll was not evident until 2–3 wk following the second atrazine application and coincided with the decrease in Chlorophyta biomass. The Chlorophyta represented over 80% of the total biomass and over 50% of the total cell num-

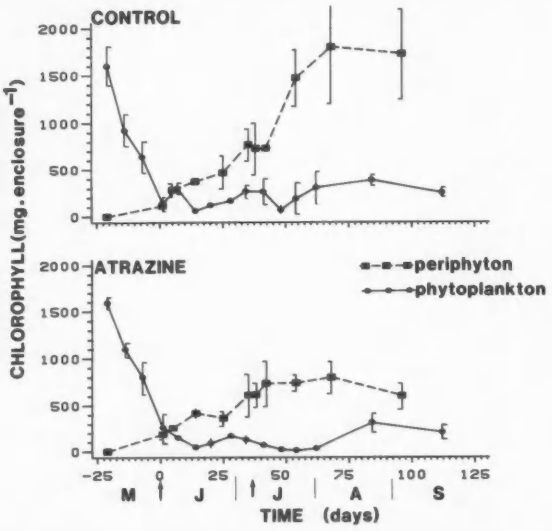


FIG. 7. Mean total chlorophyll values for periphyton and phytoplankton in the control and atrazine limnocorrals from the day periphyton colonization strips were placed in the limnocorrals. Error bars represent one standard error for the mean on each sampling day ($n = 3$). Arrows indicate atrazine treatment.

ber during the second pulse. Therefore, the major impact of atrazine on the periphyton was the decrease in the Chlorophyta. The Bacillariophyceae were the least sensitive algal group and maintained a consistently greater biomass in the atrazine limnocorrals than in the controls until day 96.

Chlorophyll, organic matter, and total algal biomass (total of Chlorophyta, Cyanophyta, and Bacillariophyceae groups) showed a 36–67% reduction in periphyton standing crop as a result of the second atrazine application. Significant reduction in dissolved oxygen and significant increases in DIC and NO_3–NO_2–N during the second pulse suggest inhibition of photosynthetic activity as well as a decreased demand for nutrients as a result of the second atrazine treatment. Water chemistry parameters are not a direct reflection of periphyton activity but also reflect the sensitivity of the phytoplankton. No recovery of the periphyton community to control levels, in terms of AFDW, chlorophyll, or algal biomass, was evident after 329 d (294 d after the second atrazine dose).

The sensitivity of the periphyton community in Lake St. George to atrazine was similar to phytoplankton sensitivity found by DeNoyelles et al. (1982), Brockway et al. (1984), and Lay et al. (1984) and falls within the range of the EC50 for simazine reported by Goldsborough and Robinson (1983). Sensitivity of the periphyton was also similar to that of four submerged vascular plant species studied by Jones and Winchell (1984). Photosynthesis in the macrophytes was inhibited by 50% when exposed to atrazine concentrations of 95 $\mu g \cdot L^{-1}$.

The results of this study were also consistent with laboratory studies on atrazine toxicity to algae (Loeppky and Tweedy 1969; Butler et al. 1975; Torres and O'Flaherty 1976; Veber et al. 1981; Stratton 1984). Laboratory studies demonstrated the immediate inhibition of photosynthesis due to atrazine treatment, but these studies did not predict the impact of long-term exposure. In the limnocorrals, the Cyanophyta were eliminated within a few weeks of atrazine exposure, while most Bacillariophyceae and Chlorophyta species remained viable even though stressed.

TABLE 3. Total limnocorral periphyton and phytoplankton net primary productivity (mg $C \cdot h^{-1} \cdot enclosure^{-1}$) for control and atrazine-treated limnocorrals in July (days 47–49).

	Control limnocorrals		Atrazine limnocorrals	
	Periphyton	Phytoplankton	Periphyton	Phytoplankton
1	2812[a]	831	593[a]	1251
2	329	474	603	1044
3	1017	2104	427	404
Mean	1719	1136	541	899

[a]Periphyton strips had colonized for 10 wk from May 6 until July 18–20.

Atrazine concentrations in the present study fall within the range at which DeNoyelles et al. (1982) and Lay et al. (1984) found that recovery was possible. At 20 and 500 μg atrazine $\cdot L^{-1}$, DeNoyelles et al. (1982) found phytoplankton succession to favour several species of flagellates which were more tolerant to the herbicide treatment. At 20 $\mu g \cdot L^{-1}$ these species played a major role in the recovery of the phytoplankton to control levels of biomass and carbon assimilation. In our study, the abundance of *Bulbochaete* sp. and *Oedogonium* sp. decreased, while *Coleochaete* sp. increased, but this species shift did not result in recovery to control levels of biomass. It is possible that some Chlorophyta species have a greater metabolic resistance to atrazine. Recent ultrastructural and biochemical investigations of the genus *Coleochaete* have revealed a close relationship between members of this genus and the ancestors of land plants (Graham 1982). Members of this genus may have the ability to metabolize atrazine into a nontoxic form as occurs in certain land plants.

The highest recorded atrazine concentrations in rivers draining agricultural land range between 26.9 and 31.7 $\mu g \cdot L^{-1}$ (Muir et al. 1978; Frank and Sirons 1979) and usually coincide with herbicide spraying season and heavy rainfalls. These atrazine concentrations are below the level which clearly impacted the Chlorophyta in our study, but they may negatively impact the Cyanophyta. The Cyanophyta were not of major importance to total algal biomass, but the heterocyst-bearing species could make a significant contribution to nutrient cycling when available inorganic nitrogen is limiting.

The inhibition of photosynthetic activity by atrazine was considered by Plumley and Davis (1980) to be analogous to the inhibition of photosynthesis by light-limiting conditions. The results of the present study support this view. During the second pulse, Chlorophyta biomass levels in the atrazine limnocorrals were almost equal over the four sampling depths. Chlorophyta biomass in the control enclosures decreased with depth, probably due to light attenuation which was found to be 76% from 0.5 to 3.5 m during the productivity measurement period. The similarity of control biomass at 3.5 m to the biomass over all depths in the atrazine limnocorrals would suggest that light attenuation had the same degree of effect as the herbicide treatment. Bacillariophyceae biomass in the control limnocorrals was unaffected by depth and contributed a greater amount to total algal biomass with increased sampling depth. As suggested by Plumley and Davis (1980), the ability of Bacillariophyceae to maintain cellular integrity under low light conditions may impart to these organisms a greater tolerance to atrazine toxicity.

In the controls, Chlorophyta biomass and periphyton organic matter values decreased with increased depth of sampling. The fact that total chlorophyll was not affected by depth may be due to the ability of algae to compensate for low light conditions by increasing the chlorophyll concentration per unit dry weight of the cell (Round 1965).

Kemmerer (1968), Klussmann and Inglis (1968), and Lund (1972) reported that periphyton build-up and movement of periphytic material into the open water of enclosures can interfere with the examination of planktonic processes within the enclosures. For the limnocorrals, periphyton biomass was greater than that of the phytoplankton within 4–5 wk of periphyton colonization, and periphyton productivity could equal that of the phytoplankton within 10 wk of periphyton colonization. Our study supports the suggestion by Lund (1972) that, in terms of primary production, the impact of mechanical enclosure of a column of water was to change the conditions within the enclosure and create an environment more similar to that of a small pond.

The impact of periphyton build-up on the ability of the limnocorrals to represent natural lake conditions is not completely known. A tendency towards a nonrandom distribution of zooplankton in limnocorrals was found by Stephenson et al. (1984) and could be due to an edge effect created by the periphyton. Periphyton build-up may also result in the removal of pesticides from the water column through adsorption, thus reducing the impact of the pesticide on the system. Further study is needed on this issue although it is clear that periphyton build-up is a factor to be considered when using limnocorrals.

Acknowledgements

We thank P. Hamilton and Dr. J. Gerrath for their advice in algal taxonomy and critical review of this work. The typing expertise was supplied by H. Daniecki. The research was supported by the Ontario Ministry of Agriculture and Food and a strategic grant from the Natural Sciences and Engineering Research Council of Canada to Drs. F. L. McEwen, N. K. Kaushik, K. R. Solomon, C. T. Corke, G. R. Stephenson, and L. V. Edgington.

References

American Public Health Association. 1976. Standard methods for the examination of water and waste water. American Public Health Association, Washington, DC.

Arvola, L. 1981. Spectrophotometric determination of chlorophyll *a* and phaeopigments in ethanol. Ann. Bot. Fenn. 18: 221–227.

Brockway, D. L., P. Smith, and F. E. Stancil. 1984. Fate and effects of atrazine in small aquatic microcosms. Bull. Environ. Contam. Toxicol. 32: 345–353.

Butler, G. L., T. R. Deason, and J. C. O'Kelly. 1975. The effect of atrazine, 2,4-*D*, methoxychlor, carbaryl and diazinon on the growth of planktonic algae. Br. Phycol. J. 10: 371–376.

Canadian Centre for Toxicology. 1984. Agricultural chemicals and farm health and safety. A background paper prepared for the Ontario task force on health and safety in agriculture. (Available from the Canadian Centre for Toxicology, 640 Gordon St., Guelph, Ont. N1G 2W1).

DeNoyelles, F., W. D. Kettle, and D. E. Sinn. 1982. The responses of plankton communities in experimental ponds to atrazine, the most heavily used pesticide in the United States. Ecology 65: 1285–1293.

Frank, R., and G. J. Sirons. 1979. Atrazine: its use in corn production and its loss to stream waters in Southern Ont., 1975–1977. Sci. Total Environ. 12: 223–239.

Goldsborough, L. G., and G. G. C. Robinson. 1983. The effect of two triazine herbicides on the productivity of freshwater marsh periphyton. Aquat. Toxicol. 4: 95–112.

Graham, L. E. 1982. The occurrence, evolution, and phylogenetic significance of parenchyma in Coleochaete Breb. (Chlorophyta). Am. J. Bot. 69: 447–454.

Jones, T. W., W. M. Kemp, J. C. Stevenson, and J. C. Means. 1982. Degradation of atrazine in estuarine water/sediment systems and soils. J. Environ. Qual. 11: 632–638.

Jones, T. W., and L. Winchell. 1984. Uptake and photosynthetic inhibition by atrazine and its degradation products on four species of submerged vascular plants. J. Environ. Qual. 13: 243–247.

Kaushik, N. K., G. L. Stephenson, K. R. Solomon, and K. E. Day. 1985. Impact of permethrin on zooplankton communities in limnocorrals. Can. J. Fish. Aquat. Sci. 42: 77–85.

Kemmerer, A. J. 1968. A method of determining fertilization requirements of a small fishing lake. Trans. Am. Fish Soc. 97: 425–428.

Klussmann, W. G., and J. M. Inglis. 1968. Polyethylene tubes for studies of fertilization and productivity. Proc. S. East. Assoc. Game Fish Comm. 22: 415–425.

Lay, J. P., A. Muller, L. Peichl, W. Klein, and F. Korte. 1984. Longterm effects of the herbicide atrazine and dichlobenil upon the phytoplankton density and physio-chemical conditions in compartments of a freshwater pond. Chemosphere 13: 821–832.

Lean, D. R. S., and B. K. Burnison. 1979. An evaluation of errors in the ^{14}C method of primary production measurement. Limnol. Oceanogr. 24: 917–928.

Loeppky, C., and B. G. Tweedy. 1969. Effects of selected herbicides upon growth of soil algae. Weed Sci. 17: 110–113.

Lund, J. W. G. 1972. Preliminary observations on the use of large experimental tubes in lakes. Verh. Int. Ver. Limnol. 18: 71–77.

Lund, J. W. G., C. Kipling, and E. D. LeCren. 1958. The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. Hydrobiologia 11: 143–170.

Lynch, T. R., H. E. Johnson, and W. J. Adams. 1982. The fate of atrazine and hexachlorobiphenyl isomer in naturally-derived model stream ecosystem. Environ. Toxicol. Chem. 1: 179–192.

Marker, A. F. H., E. A. Nusch, H. Rai, and B. Riemann. 1980. The measurement of photosynthetic pigments in freshwater and standardization of methods: conclusions and recommendations. Arch. Hydrobiol. Beih. Ergebn. Limnol. 14: 91–106.

Moreland, D. E. 1980. Mechanisms of action of herbicides. Annu. Rev. Plant Physiol. 31: 597–638.

Muir, D. C. G., J. Y. Yoo, and B. E. Baker. 1978. Residues of atrazine and *N*-deethylated atrazine in water from five agricultural watersheds in Quebec. Arch. Environ. Contam. Toxicol. 7: 221–235.

Nusch, E. A. 1980. Comparison of different methods of chlorophyll and phaeopigment determination. Arch. Hydrobiol. Beih. Ergebn. Limnol. 14: 14–36.

Plumley, F. G., and D. E. Davis. 1980. Effects of photosynthesis inhibitor atrazine on salt marsh edaphic algae in culture, microecosystems and in the field. Estuaries 3: 271–277.

Round, F. E. 1965. The ecology of algae. Cambridge University Press, Cambridge.

Solomon, K. R., K. Smith, G. Guest, J. Y. Yoo, and N. K. Kaushik. 1980. Use of limnocorrals in studying the effects of pesticides in the aquatic ecosystem. Can. Tech. Rep. Fish. Aquat. Sci. 975: 1–9.

Solomon, K. R., K. Smith, and G. L. Stephenson. 1982. Depth integrating samplers for use in limnocorrals. Hydrobiologia 94: 71–75.

Solomon, K. R., J. Y. Yoo, D. Lean, N. K. Kaushik, K. E. Day, and G. L. Stephenson. 1985. Dissipation of permethrin in limnocorrals. Can. J. Fish. Aquat. Sci. 42: 70–76.

Stephenson, G. L., P. Hamilton, N. K. Kaushik, J. B. Robinson, and K. R. Solomon. 1984. Spatial distribution of plankton in enclosures of three sizes. Can. J. Fish. Aquat. Sci. 41: 1048–1054.

Stratton, G. W. 1984. Effects of the herbicide atrazine and its degradation products, alone and in combination, on phototrophic microorganisms. Arch. Environ. Contam. Toxicol. 13: 35–42.

Torres, A. M. R., and L. M. O'Flaherty. 1976. Influence of pesticides on *Chlorella*, *Chlorococcum*, *Stigeoclonium* (Chlorophyceae), *Tribonema Vaucheria*, (Xanthophyceae) and *Oscillatoria* (Cyanophyceae). Phycologia 15: 25–36.

Veber, K., J. Zahradnik, I. Breyl, and F. Kredl. 1981. Toxic effects and accumulation of atrazine in algae. Bull. Environ. Contam. Toxicol. 27: 872–876.

Vollenweider, R. A. 1969. A manual on methods for measuring primary production in aquatic environments. IBP Handb. No. 12.

Yoo, J. Y., and K. R. Solomon. 1981. Persistence of permethrin, atrazine and methoxychlor in a natural lake system. Can. Tech. Rep. Fish. Aquat. Sci. 1151: 164–167.

Biomass and Production of Fishes in Natural and Channelized Streams

Cameron B. Portt, Eugene K. Balon,[1] and David L. G. Noakes

Department of Zoology, Group for Advancement of Fish Studies (GAFS), University of Guelph, Guelph, Ont. N1G 2W1

Portt, C. B., E. K. Balon, and D. L. G. Noakes. 1986. Biomass and production of fishes in natural and channelized streams. Can. J. Fish. Aquat. Sci. 43: 1926–1934.

Fish biomass and production were estimated at two channelized and two unchannelized stream sections. Biomass and total and available production were much lower in the channelized sections than in the natural sections. Regressions of the log of production versus the log of initial biomass had slopes significantly different from 1. This is apparently due to changes in age structure which occurred concurrently with changes in biomass. The physical homogeneity of channelized streams provides opportunities for manipulative research.

Nous avons calculé la biomasse et la production de poisson dans deux portions de cours d'eau canalisées et deux portions non canalisées. La biomasse, la production totale et la production disponible étaient beaucoup plus basses dans les portions canalisées que dans les autres. Les régressions pratiquées sur le logarithme de la production par opposition au logarithme de la biomasse initiale donnaient des courbes nettement différentes de 1. Ce phénomène est apparemment dû à des modifications dans la structure des âges qui se sont produites en même temps que des changements de la biomasse. L'homogénéité physique des cours d'eau canalisés permet de procéder à des manipulations.

Received January 4, 1984
Accepted June 24, 1986
(J7651)

Reçu le 4 janvier 1984
Accepté le 24 juin 1986

Streams in southern Ontario are often modified to benefit agriculture. One common modification is straightening of the stream channel and removal of any obstructions to flow, a practice commonly referred to as channelization. The impact of stream channelization on fish communities is assumed to be negative (Griswold et al. 1982) but few studies have quantitatively examined the effect of channelization on stream fish communities, and those which have (Congdon 1971; Bayless and Smith 1967) have only determined the effect on fish biomass. In this study we compare fish production and biomass and their interrelationships in channelized and unchannelized stream segments.

Other researchers have estimated fish production in streams which varied in physical characteristics (Lotrich 1973; O'Connor and Power 1976; Small 1975). Their hypothesis was that the rate of fish production would be different in physically different habitats, and indeed this proved to be the case. However, it is also important to know if fish production is similar in physically similar habitats (Mahon and Balon 1985; Watson and Balon 1984). The physical similarity between the two channelized sites also allowed us to test this frequently unstated assumption.

Materials and Methods

Study Area

Mud Creek and North Branch Creek both arise in Oxford County, Ontario, and have average gradients of 0.1 and 0.2%, respectively (Department of Energy, Mines and Resources 1972). They join (43°08′N, 80°54′W) to form the Middle Thames River which ultimately drains into Lake St. Clair. The underlying bedrock of Silurian and Devonian sediments is overlain by glacial drift of varying texture and thickness (Wickland and Richards 1961). Land use within the watershed is predominantly agricultural. The lower 8 km of Mud Creek was channelized in 1971, 6 yr prior to the beginning of this study, time sufficient for recolonization and stabilization of the fish community (Griswold et al. 1982).

Fish biomass and production were estimated at two locations within the channelized portion of Mud Creek (MC2, MC3) and at two locations which had not been channelized on Mud (MC1) and North Branch creeks (NBC, Fig. 1). The sampling sites were bordered by abandoned fields and pastures except at MC2 where a partially wooded buffer strip occurred between the north bank of the creek and a hay field. The substrate at MC1 was predominantly silt with small amounts of sand and occasional boulders. There were no riffles and only one distinct pool within the section. Sites MC2 and MC3, which were within the channelized portion of Mud Creek, exhibited little within-site variation in depth and had sand substrate. The upstream portion of site NBC was a riffle with gravel and rubble substrate whereas the lower portion was a run with sand substrate.

At each site, transects were taken at 2-m intervals along a base line which was parallel to the stream. The distances to both banks and to each 10-cm depth increment were measured and the maximum depth was determined along each transect. These coordinates were plotted and a bathymetric map of each site was prepared from which the area, volume, and mean depth of each site was determined (Table 1).

[1] Author to whom all correspondence should be addressed.

TABLE 1. Physical characteristics of the four sites.

Site	MC1	MC2	MC3	NBC
Date	June 7, 1977	June 5, 1977	July 5, 1977	July 8, 1977
Length (m)	112	92	70	58
Mean width (m)	3.3	4.9	7.2	6.5
Area (m^2)	368	454	505	376
Mean depth (cm)	35	15	11	13
Maximum depth (cm)	64	34	40	40
Mean surface velocity ($m \cdot s^{-1}$)	0.12	0.24	0.22	0.36

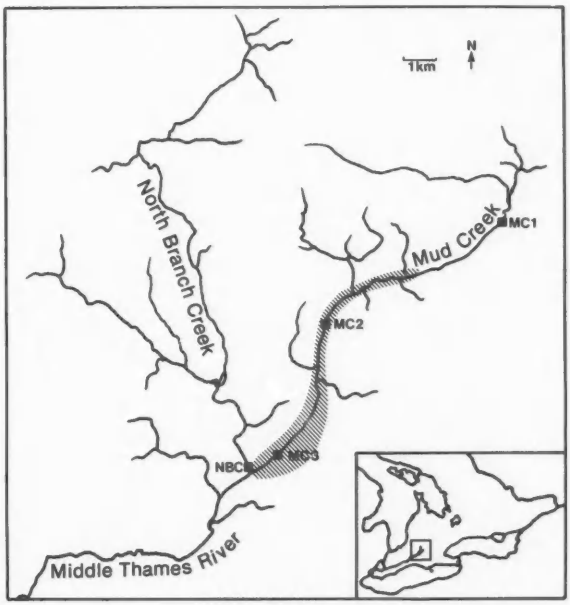


FIG. 1. Study area.

Collection of Fishes

At each location the designated section of stream was blocked off using 3-mm circular-mesh netting. Each site was fished seven to nine times using a Smith-Root type VI electrofisher which delivered pulsed DC current at 60 pps and 560 V. The duration of each fishing and of rest periods between fishings was kept constant at each site. Following capture, all fish were preserved in 10% formalin. They were identified several weeks later using keys in Scott and Crossman (1973) and Trautman (1957). For each species the standard length (SL) of all individuals was determined to the nearest millimetre and their total weight was determined to the nearest gram.

Density Estimates and Determination of Age, Growth, and Sex

Because effort was kept constant between fishings at each location, the simplification of Leslie's method of relating catch and cumulative catch (Leslie and Davis 1939; Ricker 1975) was used to estimate fish abundance. The abundance of species which exhibited a wide size range was estimated separately for different length classes (DeLury 1947; Sullivan 1956; Stewart 1975).

Subsamples of 5 (MC2, MC3) or 10 (MC1, NBC) individuals were taken from each millimetre length group of the species for which production was calculated, where possible. If fewer individuals were present, all individuals in that length group were processed. Each fish so selected was rinsed in water and blotted dry. Fish weighing less than 1 g were weighed to the nearest 0.01 g; fish weighing more than 1 g were weighed to the nearest 0.1 g. Four key scales (Segerstråle 1933) were removed from each individual in the subsample and mounted dry between glass slides. The body cavity was then opened and gonads which could not be sexed by eye were examined microscopically. Fish which could not be sexed in this fashion were considered juveniles. The proportion of mature females in each size group was determined.

Scales were examined and an age was assigned based on the number of annuli observed. Some older fish had not yet formed an annulus in the year of collection. If the distance between the last annulus and the scale margin was large relative to the distance between the last two annuli, an age 1 yr greater than the number of annuli was assigned (Balon 1972, 1974). The distance from the focus to the scale margin and to each annulus was measured. If an individual had not yet formed an annulus in the current year the scale margin was considered to be the current year's annulus. Aging was validated by the examination of length–frequency distributions for all species.

Total and available production were calculated from the time of spawning using the algebraic method (Ricker 1946; Balon 1974) as outlined in Mahon and Balon (1977). At sites MC1 and MC2 the time of collection coincided with the time of spawning for all species except *Catostomus commersoni*. Therefore, the estimated abundance and the mean weight of each age group at the time of collection were used in the production calculations.

For sites MC3 and NBC and for *C. commersoni* at MC1, spawning preceded collection by approximately 1 mo. The number of fish in any age group at the time of spawning (N_i) was estimated by adjusting the number in that age group at the time of collection (N_{i+}) using the formula $N_i = N_{i+} e^{Zt}$, where Z is the instantaneous rate of change in numbers and t is the fraction of a year between the time of collection and of spawning, in this case 1/12.

Examination of scales from fishes collected at MC1 and MC2 indicated that the younger age classes had grown considerably during the current year (since annulus formation) although many of the older individuals had not yet formed the current year's annulus. Therefore, a correction factor was calculated for each age class by expressing the difference between the mean scale radius and the mean distance to the current year's annulus as a percentage of the former (see Portt and Balon 1984). These correction factors were used to estimate the scale radius at the time of spawning for species at MC3

TABLE 2. Estimated number ($\hat{N}$) and biomass ($\hat{B}$) present at the time of collection and percentages of total in parentheses.

Species	MC1		MC2		MC3		NBC	
	$\hat{N}$	$\hat{B}$ (g)	$\hat{N}$	$\hat{B}$ (g)	$\hat{N}$	$\hat{B}$ (g)	$\hat{N}$	$\hat{B}$ (g)
Notropis cornutus	929 (25.8)	4 335 (31.8)	161 (7.2)	206 (5.2)	840 (43.3)	1 616 (46.2)	991 (20.2)	3 433 (30.5)
Semotilus atromaculatus	486 (13.5)	3 024 (21.8)	385 (17.3)	1 412 (35.9)	104 (5.4)	599 (17.1)	32 (0.7)	499 (4.4)
Rhynichthys atratulus	333 (9.2)	821 (5.9)	857 (38.6)	1 614 (41.0)	203 (10.5)	431 (12.3)	622 (12.7)	1 517 (13.5)
Nocomis spp.	18 (0.5)	95 (0.7)	1 (—)	1 (—)	85 (4.4)	164 (4.7)	370 (7.5)	1 308 (11.6)
Etheostoma nigrum	996 (27.6)	870 (6.3)	340 (15.3)	392 (10.0)	299 (15.4)	412 (11.8)	47 (1.0)	67 (0.6)
E. microperca	102 (2.8)	30 (0.2)	151 (6.8)	43 (1.1)	204 (10.5)	67 (1.9)	240 (4.9)	79 (0.7)
Pimephales notatus	161 (4.5)	537 (3.9)	19 (0.9)	7 (0.2)	100 (5.2)	93 (2.7)		
Culaea inconstans	281 (7.8)	245 (1.8)	283 (12.7)	227 (5.8)	91 (4.7)	84 (2.4)		
Notropis rubellus	2 (0.1)	5 (—)			1 (—)	2 (—)	400 (8.1)	688 (6.1)
Etheostoma caeruleum			6 (0.3)	8 (0.2)	4 (0.2)	9 (0.3)	1 092 (22.2)	1 509 (13.4)
E. flabellare			15 (0.7)	10 (0.3)	6 (0.3)	11 (0.3)	820 (16.7)	864 (7.7)
Pimephales promelas	147 (4.1)	674 (4.9)	1 (—)	4 (0.1)				
Umbra limi			3 (0.1)	11 (0.3)	2 (0.1)	14 (0.4)		
Hypentelium nigricans	1 (—)	38 (0.3)					10 (0.2)	261 (2.3)
Catostomus commersoni	146 (4.1)	3 161 (22.8)						
Lepomis gibbosus	1 (—)	45 (0.3)						
Etheostoma blennoides							211 (4.3)	617 (5.5)
Ambloplites rupestris							1 (—)	13 (0.1)
Notropis hudsonius							18 (0.4)	33 (0.3)
N. volucellus							50 (1.0)	377 (3.3)
Noturus flavus							9 (0.2)	6 (0.1)
Totals	3 603	13 880	2 222	3 935	1 939	3 501	4 931	11 271
Totals·m^{-2}	9.8	37.7	4.9	8.7	3.8	6.9	13.1	30.0
Mean weight of an individual (g)	3.8		1.8		1.8		2.3	

and NBC. The mean of the correction factors calculated for cyprinids at MC1 and MC2 was used for *Notropis rubellus* and *Nocomis* spp. The mean correction factors for age 1 *Etheostoma nigrum* were used for age 1+ *Etheostoma blennioides*, *E. caeruleum*, and *E. flabellare*.

For each age a weighted mean length was calculated from the mean back-calculated lengths at that age for all age classes present and the estimated number of fish in each age class. Mean weights were estimated by substituting weighted mean standard lengths into the appropriate weight versus standard length equations (Mahon and Balon 1977; Mahon et al. 1979; Halyk and Balon 1983). This method can result in mean weights being underestimated (Nielson and Schoch 1980); however, the mean error in this study was small (<5%), as the coefficient of variation in length was less than 0.1 for most age classes.

Population fecundity, estimated by multiplying the number of mature females by mean individual fecundity, was used as an estimate of density at age 0. If ripe females were available the ovaries of selected individuals were removed and fecundity was determined either by direct count or gravimetrically (McGregor 1922). If no ripe females were available, fecundities were obtained from the literature. The sex ratio was assumed to be 1:1 at time 0. The mean weight of a mature egg, determined to the nearest milligram, was 1 mg for *Notropis cornutus*, *Pimephales promelas*, *P. notatus*, *Etheostoma microperca*, and *E. nigrum* and 2 mg for *Rhinichthys atratulus* and *Semotilus atromaculatus*. Production was calculated for species from which ripe females were not available using an egg weight of 1 mg with the exception of *Nocomis* spp. for which a weight of 2 mg was used. The estimated weight of eggs produced was added to estimates of total and available production for species which had spawned prior to collection.

Analysis of covariance was used to examine the relationships between production and initial biomass at the four sites.

Results

Twenty-two species were encountered at one or more sites. All individuals were identified to species except juveniles of the genus *Nocomis*. Mature males of *N. micropogon* and *N. biguttatus* were identified based on the distribution of breeding tubercles (Scott and Crossman 1973) but juveniles of the two species could not be distinguished (see Ferguson et al. 1981). These two species were therefore treated as one in subsequent analyses. Twelve species were captured at each of the channelized sites, and 13 and 15 species were captured at MC1 and NBC, respectively (Table 2).

Total abundance, biomass, and mean weight were lower at the channelized sites. Cyprinids dominated in terms of both numbers and biomass at three of the four sites (Table 2). At NBC, darters and cyprinids were present in nearly equal numbers but cyprinids accounted for 70% of the biomass. *Catostomus commersoni*, which was only captured at MC1, accounted for nearly 23% of the biomass at that location. The species for which production was calculated made up 97.4, 93.7, 96.7, and 89.5% of the estimated biomass at the time of collection at MC1–NBC respectively.

The proportion of mature females at each age varied between sites (Table 3). In particular, a greater proportion of young *R. atratulus* and *E. nigrum* were mature at NBC than at the other locations. The mean fecundities, which were used to estimate the density of each species at age zero, are listed in Table 4.

TABLE 3. Percentage of females at each age which were sexually mature.

Species	Site	% mature at each age			
		1	2	3	4
Notropis cornutus	MC1	29	93	100	
	MC2	0	97		
	MC3	1	79	100	
	NBC	15	98	100	100
Notropis rubellus	NBC	65	100	100	
Nocomis spp.	MC3	0	33	100	
	NBC	0	100	100	
Pimephales notatus	MC1	0	100	100	
	MC3	0	0		
Pimephales promelas	MC1	100	100	100	
Rhinichthys atratulus	MC1	12	93	100	
	MC2	15	98		
	MC3	21	96	100	
	NBC	73	100		
Semotilus atromaculatus	MC1	0	20	100	
	MC2	0	22	100	
	MC3	0	71	100	
Catostomus commersoni	MC1	0	0	13	67
Etheostoma blennioides	NBC	100	100	100	
Etheostoma caeruleum	NBC	100	100	100	100
Etheostoma flabellare	NBC	98	100		
Etheostoma microperca	MC2	100	100	100	
	MC3	100	100		
	NBC	100	100		
Etheostoma nigrum	MC1	6	100	100	
	MC2	27	90		
	MC3	26	79	100	
	NBC	100	100		

Initial and mean biomass and available and total production were much lower at the two channelized sites (Table 5). *Rhinichthys atratulus* accounted for the most biomass and production at MC2, while *N. cornutus* was the dominant species at the other sites. At NBC, *E. caeruleum* made the second largest contribution to biomass and production. *Semotilus atromaculatus* was the second largest contributor at the other sites.

The ratios of $P_T:B_I$ and $P_T:\bar{B}$ varied in a similar fashion, being largest for short-lived species such as *E. microperca*. These ratios varied considerably between sites for individual species. The ratios for *P. promelas* at MC3 are anomalously low because production was only calculated for the second year of life.

Plots of P_T and P_A versus B_I indicated that variance increased as B_I increased. Therefore, all analyses of covariance were conducted on log-transformed data (the data for *P. notatus* at MC3 were excluded). The slopes of regressions of log P_T versus log B_I did not differ significantly between sites ($P > 0.36$). Calculations with a common slope indicated that the intercepts did differ significantly between sites ($P < 0.001$). Neither the slopes nor the intercepts of regressions of log P_A versus log B_I differed significantly between sites ($P > 0.83$ and $P > 0.20$, respectively). The slopes of regressions of log P_A versus log P_T did not differ significantly between sites ($P > 0.64$) but the intercepts did ($P < 0.001$). Slopes of regressions of log P_T versus log B_I and log P_A versus log B_I differed significantly from 1.0, while the slope of the log P_A versus log P_T regressions did not. The regression equations, expressed as power functions, are presented in Table 6.

Discussion

Biomass

The estimates of biomass at the two channelized sites are at the low end of the range reported for warmwater streams in southern Ontario, while the estimates of biomass at the unchannelized sites are comparable with the average values reported in other studies. Mahon and Balon (1985) reported that biomass ranged from 174 to 462 $kg \cdot ha^{-1}$ (mean = 296) in six streams. Portt (1985) determined biomass at 18 locations to range from 56 to 2473 $kg \cdot ha^{-1}$. In that study the mean of 393 $kg \cdot ha^{-1}$ was reduced to 270 $kg \cdot ha^{-1}$ when the highest value was excluded. Other estimates include 214 $kg \cdot ha^{-1}$ for Irvine Creek, Ontario (Halyk and Balon 1983), and 32, 33, and 61 $kg \cdot ha^{-1}$ at three locations in the Speed River, Ontario (Mahon et al. 1979).

Our results are consistent with those of Congdon (1971) who estimated fish biomass in unchannelized and channelized sections of the Chariton River, Missouri, to be 341 and 59 $kg \cdot ha^{-1} \cdot yr^{-1}$, respectively. Bayless and Smith (1967) reported that biomass was much lower in channelized streams than in natural streams in North Carolina. In contrast, Schlosser (1982) found biomass to be higher in a channelized stream than in an unchannelized stream on five of six sampling dates. However, in that study there were other factors which differed between the two streams, most notably the presence of riparian vegetation along the unchannelized stream and increased nutrient levels in the channelized stream due to municipal wastewater inputs.

Factors suggested to be responsible for reducing fish biomass in channelized streams include reduced space, loss of riffles, decreased habitat diversity, and habitat instability in terms of substrate, water levels, and water temperature (Congdon 1971).

Portt (1985) determined biomass in pools and raceways at 18 locations in unchannelized streams in southern Ontario and found that mean depth explained 74% of the variation in biomass. Comparison of those results with the results of this study (Fig. 2) show excellent agreement for the three raceway-pool sites in Mud Creek. This suggests that for nonriffle habitats the primary cause of biomass reduction following channelization is the reduction in depth. Site NBC, which was more than 50% riffle, clearly supported a much greater biomass than nonriffle habitats of similar depth.

Biomass is the product of abundance and mean weight and both of these were lower at the two channelized sites. At MC1, both abundance and mean weight were 2–2.5 times that at the channelized sites, while at NBC the mean weight was only slightly greater but abundance was considerably higher.

Portt (1985) reported that the logarithm of abundance was significantly correlated with depth ($P < 0.05$, $r^2 = 0.485$).

TABLE 4. Mean fecundity for all species for which production was calculated.

Species	Source		Mean fecundity
Notropis cornutus	Gravid females; MC2		665
Pimephales notatus	Gravid females; MC1		693
Pimephales promelas	Gravid females; MC1		753
Rhinichthys atratulus	Gravid females; MC1		264
Semotilus atromaculatus	Gravid females; MC1 and MC2		774
Etheostoma microperca	Gravid females; MC2		85
Etheostoma nigrum	Gravid females; MC2		177
Notropis rubellus	Pfeiffer 1955	1 yr	600
		2 yr	1 090
		3 yr	1 175
Nocomis spp.	Lachner 1952		553
Catostomus commersoni	Scott and Crossman 1973		20 000
Etheostoma blennioides	Fahey 1954	1 yr	441
		2 yr	796
		3 yr	1 502
		4 yr	1 832
Etheostoma caeruleum	Approximation from Williams 1959		200
Etheostoma flabellare	Lake 1936		226

TABLE 5. Initial biomass (B_I), mean biomass ($\bar{B}$), total annual production (P_T), and available annual production (P_A).

Species	B_I (kg·ha^{-1})	$\bar{B}$ (kg·ha^{-1})	P_T (kg·ha^{-1}·yr^{-1})	P_A (kg·ha^{-1}·yr^{-1})
		Site MC1		
Notropis cornutus	117.80	96.11	129.29	76.93
Pimephales notatus	14.59	8.70	13.97	11.90
Pimephales promelas	18.32	13.56	16.06	7.39
Rhinichthys atratulus	22.31	15.33	30.68	16.09
Semotilus atromaculatus	82.17	64.38	102.39	58.91
Catostomus commersoni	68.02	56.09	82.93	42.77
Etheostoma nigrum	23.64	19.13	34.43	17.80
Total	346.85	273.30	409.75	231.79
		Site MC2		
Notropis cornutus	4.54	3.30	8.52	3.94
Rhinichthys atratulus	35.55	26.28	58.46	27.36
Semotilus atromaculatus	31.10	25.57	43.74	21.61
Etheostoma microperca	0.95	0.70	2.27	0.88
Etheostoma nigrum	8.63	5.24	11.43	5.51
Total	80.77	61.09	124.42	59.30

TABLE 5. (*Concluded*)

Species	B_I (kg·ha^{-1})	$\bar{B}$ (kg·ha^{-1})	P_T (kg·ha^{-1}·yr^{-1})	P_A (kg·ha^{-1}·yr^{-1})
		Site MC3		
Notropis cornutus	34.79	24.75	62.91	28.95
Nocomis spp.	3.98	2.73	7.01	3.60
Pimephales notatus	1.70	0.50	0.18	0.02
Rhinichthys atratulus	8.14	5.96	15.21	6.74
Semotilus atromaculatus	10.46	8.34	16.59	7.68
Etheostoma microperca	2.14	1.09	4.53	1.84
Etheostoma nigrum	7.17	4.79	12.04	5.76
Total	68.38	48.16	118.47	54.59
		Site NBC		
Notropis cornutus	89.81	78.59	135.40	62.90
Notropis rubellus	19.76	15.08	54.31	16.97
Nocomis spp.	34.20	27.71	67.34	28.80
Rhinichthys atratulus	33.09	25.93	66.33	28.09
Etheostoma blennioides	15.27	12.34	30.35	10.83
Etheostoma caeruleum	42.97	36.12	84.02	30.49
Etheostoma flabellare	24.89	19.26	54.34	19.10
Etheostoma microperca	2.45	1.41	5.77	2.34
Etheostoma nigrum	1.54	1.04	2.79	1.23
Total	263.98	217.48	500.65	200.75

TABLE 6. Regression equations expressed as power functions derived from analyses of covariance. Combined r^2 for log P_T versus log B_I, log P_A versus log B_I, and log P_A versus log P_T is 0.983, 0.984, and 0.988, respectively.

Site	Regression
MC1	$P_T = 1.39 B_I^{0.95}$
MC2	$P_T = 1.88 B_I^{0.95}$
MC3	$P_T = 1.99 B_I^{0.95}$
NBC	$P_T = 2.35 B_I^{0.95}$
All	$P_A = B_I^{0.95}$
MC1	$P_A = 0.56 P_T$
MC2	$P_A = 0.45 P_T$
MC3	$P_A = 0.46 P_T$
NBC	$P_A = 0.39 P_T$

Comparison of his results with those from this study indicate that abundance at the sites in Mud Creek are comparable but that abundance at site NBC is higher than that for nonriffle sites of similar depth. A large proportion (43%) of the fishes present at NBC were *E. caeruleum*, *E. flabellare*, and *E. blenniodes*. These three darters were captured primarily in the riffle area and accounted for less than 1% of the total numbers at the other sites. If these species are excluded the abundance at NBC would be 7.4·m^{-2}, less than at MC1 but still higher than at the two channelized sites.

Mean weight (all species) was lowest at the two channelized sites. Mean weight can be influenced by species composition, age structure, and growth rates. Species composition clearly influenced mean weight at NBC where darters were abundant. Examination of mean weights for species which were common at three or four sites indicated that the mean weights of the cyprinids, especially the larger cyprinids such as *N. cornutus*, *S. atromaculatus*, and *Nocomis* spp., were greater at the un-

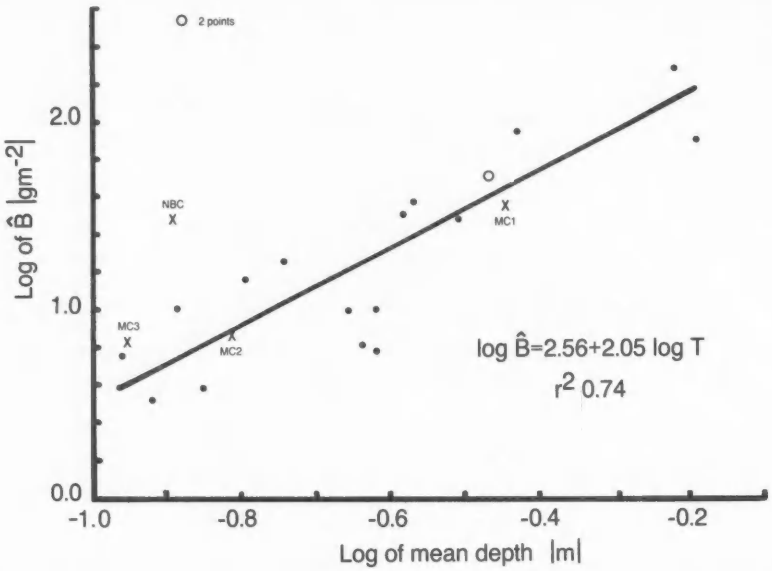


FIG. 2. Log of biomass versus the log of mean depth from this study superimposed on data from Portt (1985).

channelized sites. These differences were due primarily to the presence of older individuals at the unchannelized sites rather than to differences in growth rates between sites (Portt 1979). At MC1, which was the deepest site sampled in this study, there were more 2-yr-olds than 1-yr-olds of all five species for which production was calculated. This was not the case at the other locations. The mean weights of small species such as *E. nigrum* and *E. microperca* showed little variation between sites. These results are consistent with those of Halyk and Balon (1983) who found that age class structure, not growth rate, was the main factor influencing mean weight in floodplain pools.

The age structure of stream populations is strongly influenced by depth, as shown by Mahon and Portt (1985) who examined local size-related segregation of fishes in two streams, one of which was North Branch Creek. Gibbons and Gee (1972) reported that young *R. atratulus* inhabit shallower habitats than adults, and Moshenko and Gee (1973) reported similar findings for *S. atromaculatus*.

Production

Total and available production were also lower at the channelized sites. The similarity in the estimated production at MC2 and MC3, despite obvious differences in the relative abundance of species, supports the frequently unstated assumption that production is similar in physically similar sections of streams.

Estimates of total production in other warmwater streams in southern Ontario include 174–379 $kg \cdot ha^{-1} \cdot yr^{-1}$ ($n = 6$, mean = 279; Mahon and Balon 1985) and 298 $kg \cdot ha^{-1} \cdot yr^{-1}$ (Halyk and Balon 1983). Estimates for the Speed River, Ontario, ranged from 15 to 54 $kg \cdot ha^{-1} \cdot yr^{-1}$ (Mahon et al. 1979) but these may be underestimates due to errors in back-calculations (see Portt and Balon 1984). The estimates at MC2 and MC3 are close to the mean of 121 $kg \cdot ha^{-1} \cdot yr^{-1}$ (range 83–161) reported for Polish rivers (Mahon and Balon 1985). The estimated rates of production at MC1 and NBC exceed, to our knowledge, all other published estimates of total production for North American streams with the exception of Naiman's (1976) estimate of 1554 $kg \cdot ha^{-1} \cdot yr^{-1}$ for *Cyprinodon nevadensis* in a thermal stream. In fact, only four other fish production estimates exceed 500 $kg \cdot ha^{-1} \cdot yr^{-1}$, 547 $kg \cdot ha^{-1} \cdot yr^{-1}$ for the Horokiwi Stream (Allen 1951), 596 $kg \cdot ha^{-1} \cdot yr^{-1}$ for the River Torrant (Mann 1971), an approximation of 745 $kg \cdot ha^{-1} \cdot yr^{-1}$ for Hinaki Stream (Hopkins 1971), and the extremely high estimate of 1540 $kg \cdot ha^{-1} \cdot yr^{-1}$ for the River Thames (Mathews 1970).

It has been suggested that the ratio of production to mean biomass ($P_T:\bar{B}$) may be used to estimate production (given a knowledge of mean biomass), and this ratio has been calculated for many species of fish and invertebrates (Mathews 1970; Waters 1969, 1977; Chapman 1978). However, the determination of mean biomass requires either repeated sampling of the populations in question or the aging of individuals from a single sample in order to estimate the growth rate and rate of change in numbers. Initial biomass, on the other hand, can be determined from a single estimate of density at the time of spawning. The two ratios vary in a similar fashion, being larger for small short-lived species. In this study the range and variance of $P_T:B_I$ (0.88–2.75, $\sigma^2 = 0.203$) were less than the range and variance of $P_T:\bar{B}$ (1.18–4.16, $\sigma^2 = 0.572$). The $P_T:B_I$ ratio appears better suited to the process of predicting production from biomass data.

Regressions of production versus biomass allow statistical comparisons of intersite variation in the relationship between these parameters. Because each species is weighted equally, a more realistic characterization of the fish taxocene(s) is obtained than if totals of production and biomass are compared. The slope of the log P_T versus log B_I regressions is significantly different from 1.0 (t-test, $P < 0.05$). Humphreys (1979) reported a common slope of 0.942 for regressions of the log of production versus the log of respiration. He noted that any

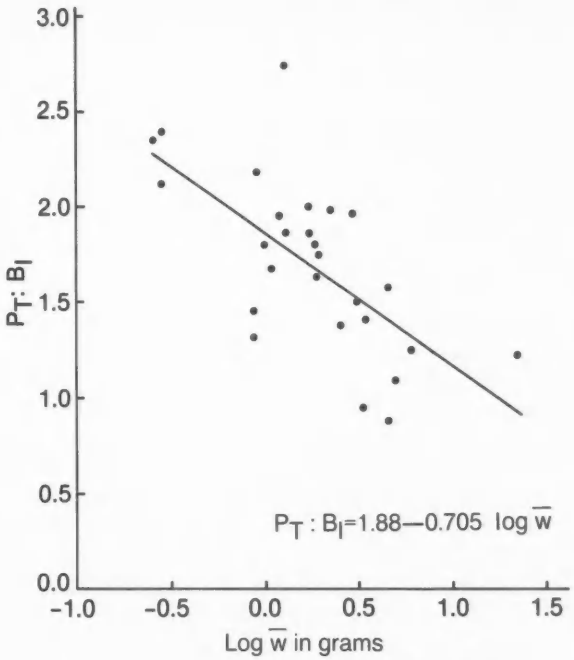


FIG. 3. $P_T:B_I$ versus mean fish weight and regression line.

departure of the slope from unity implies that the magnitude of respiration (biomass) affects the production efficiency and that there was currently no theoretical basis for using a slope different from 1.0. Banse and Mosher (1980) concluded that "annual P/B rates of entire populations do not seem to be density-dependent in any consistent manner."

In this study the decrease in the $P_T:B_I$ ratio with increasing biomass appears to be due to the aforementioned relationships between biomass and depth and age structure and depth. The $P_T:B_I$ is negatively correlated with mean fish weight (Fig. 3) because increases in mean weight result from a shift in age structure towards older individuals.

The intercepts of the regressions of total production versus initial biomass for the channelized sites fall between those calculated for the other sites. No significant differences occurred between the regressions of available production versus initial biomass for the four sites. The $P_A:B_I$ ratio is a measure of the proportion of initial biomass which is replaced (or lost) each year. This relationship did not vary between sites, indicating that the principal difference between sites is the proportion of total production which is retained or lost from that site, either through mortality or emigration, each year. Regressions of P_A versus P_T confirm this. Once again the regression coefficients for the channelized sites fell between those for the two unchannelized sites.

Analysis of covariance is a useful technique for examining the relationships between production and biomass at the taxocene level. Further research is needed to determine if the relationships identified in this study apply over a broad range of habitats and communities or alternatively if they change in a predictable manner. Factors which could be examined include nutrient additions, water temperature, and harvest or predation. It appears that the principal effect of channelization is a reduction in biomass and that the relationships between biomass and production undergo relatively less change as a result. In nonriffle habitats the reduction in biomass is due primarily to the reduction in depth which typically accompanies channelization. Where riffles are destroyed, this can also result in reduced biomass (Edwards et al. 1984).

One of the problems faced in trying to conduct manipulative research in streams is the difficulty in finding habitats which are sufficiently similar physically. This is critical given the significance of factors such as depth to stream fish communities. Based on our results, we suggest that channelized streams can provide unique opportunities to study the effects of factors such as nutrient enrichment, predation, or harvest in the absence of physical variability which can confound results.

Acknowledgments

We would like to thank Robin Mahon for his assistance and criticism which was given freely throughout this project and Don Stevens for his advice and useful comments on the manuscript. The cooperation of Marilyn White at all stages was of great benefit. Janet Hines assisted with the statistical aspects of this study. George Dixon, Larry Halyk, Sam Katuramu, Dave Maraldo, Rob MacGregor, Susan Mahon, Jim McElman, Mike Paine, and Tadeusz Penczak all assisted in a variety of ways. The study was funded by Natural Sciences and Engineering Research Council of Canada grants to E.K.B.

References

ALLEN, K. R. 1951. The Horokiwi Stream: a study of a trout population. Fish Bull. N.Z. Mar. Dep. 10: 1–238.

BALON, E. K. 1972. Possible stock size assessment and available production survey as developed on Lake Kariba. Afr. J. Trop. Hydrobiol. Fish. 2: 45–73.

1974. Fish production of a tropical ecosystem, p. 249–676. *In* E. K. Balon and A. G. Coche [ed.] Lake Kariba: a manmade tropical ecosystem in Central Africa. Monogr. Biol. 24. Dr W. Junk Publishers, The Hague.

BANSE, K., AND S. MOSHER. 1980. Adult body mass and annual production/biomass relationships of field populations. Ecol. Monogr. 50: 355–379.

BAYLESS, J., AND W. B. SMITH. 1967. The effect of channelization upon the fish populations of lotic waters in eastern North Carolina. Proc. 18th Annu. Conf. Southeast. Assoc. Game Fish Comm.: 230–238.

CHAPMAN, D. W. 1978. Production in fish populations, p. 5–25. *In* S. D. Gerking [ed.] Ecology of freshwater fish production. Blackwell Scientific Publications, Oxford.

CONGDON, J. C. 1971. Fish populations of channelized and unchannelized sections of the Chariton River, Missouri. *In* E. Schneberger and J. L. Funk [ed.] Stream channelization: a symposium. Spec. Publ. No. 2, North Central Division, American Fisheries Society.

DELURY, D. B. 1947. On the estimation of biological populations. Biometrics 3: 145–167.

DEPARTMENT OF ENERGY, MINES AND RESOURCES. 1972. Surveys and Mapping Branch. Topographic map series. Woodstock 40 P/2 and Stratford 40 P/7.

EDWARDS, C. J., B. L. GRISWOLD, R. A. TUBB, E. C. WEBER, AND L. C. WOODS. 1984. Mitigating effects of artificial riffles and pools on the fauna of a channelized warmwater stream. North Am. J. Fish. Manage. 4: 194–203.

FAHEY, W. E. 1954. The life history of the northern greenside darter, *Etheostoma blennioides blennioides* Rafinesque. J. Elisha Mitchell Sci. Soc. 70: 139–205.

FERGUSON, M. M., D. L. G. NOAKES, AND R. G. DANZMAN. 1981. Morphological and biochemical systematics of chubs, *Nocomis biguttatus* and *N. micropogon* (Pisces: Cyprinidae), in southern Ontario. Can. J. Zool. 59: 771–775.

GIBBONS, J. R. H., AND J. H. GEE. 1972. Ecological segregation between longnose and blacknose dace (genus *Rhinichthys*) in the Mink River, Manitoba. J. Fish. Res. Board Can. 29: 1245–1252.

GRISWOLD, B. L., C. J. EDWARDS, AND L. C. WOODS, III. 1982. Recolonization of macrovertebrates and fish in a channelized stream after drought.

Ohio J. Sci. 82: 96–102.

HALYK, L. C., AND E. K. BALON. 1983. Structure and ecological production of the fish taxocene of a small floodplain system. Can. Z. Zool. 61: 2446–2464.

HOPKINS, C. L. 1971. Production of fish in two small streams in the north island of New Zealand. N.Z. J. Mar. Freshwater Res. 5: 280–290.

HUMPHREYS, W. F. 1979. Production and respiration in animal populations. J. Anim. Ecol. 48: 427–453.

LACHNER, E. A. 1952. Studies of the biology of the cyprinid fishes of the chub genus *Nocomis* of northeastern United States. Am. Midl. Nat. 48: 433–466.

LAKE, C. T. 1936. The life history of the fan-tailed darter, *Catonotus flabellaris flabellaris* (Rafinesque). Am. Midl. Nat. 17: 816–830.

LESLIE, P. H., AND D. H. S. DAVIS. 1939. An attempt to determine the absolute number of rats on a given area. J. Anim. Ecol. 8: 94–113.

LOTRICH, V. A. 1973. Growth, production, and community composition of fishes inhabiting a first-, second-, and third-order stream of eastern Kentucky. Ecol. Monogr. 43: 377–397.

MAHON, R., AND E. K. BALON. 1977. Ecological fish production in Long Pond, a lakeshore lagoon on Long Point, Lake Erie. Environ. Biol. Fishes 2: 261–284.

1985. Fish production in warmwater streams in Poland and Ontario. Can. J. Fish. Aquat. Sci. 42: 1211–1215.

MAHON, R., E. K. BALON, AND D. L. G. NOAKES. 1979. Distribution, community structure and production of fishes in the upper Speed River, Ontario: a preimpoundment study. Environ. Biol. Fishes 4: 219–244.

MAHON, R., AND C. B. PORTT. 1985. Local size related segregation of fishes in streams. Arch. Hydrobiol. 103: 267–271.

MANN, R. H. K. 1971. The populations, growth and production of fish in four small streams in southern England. J. Anim. Ecol. 40: 155–190.

MATHEWS, C. P. 1970. Estimates of production with reference to general surveys. Oikos 21: 129–133.

MCGREGOR, E. A. 1922. Observations on the egg yield of Klamath River king salmon. Calif. Fish Game 8: 160–164.

MOSHENKO, R. W., AND J. H. GEE. 1973. Diet, time and place of spawning, and environment occupied by creek chub (*Semotilus atromaculatus*) in the Mink River, Manitoba. J. Fish. Res. Board Can. 30: 347–362.

NAIMAN, R. J. 1976. Productivity of a herbivorous pupfish population (*Cyprinodon nevadensis*) in a warm desert stream. J. Fish Biol. 9: 125–137.

NIELSON, L. A., AND W. F. SCHOCH. 1980. Errors in estimating mean weight and other statistics from mean length. Trans. Am. Fish. Soc. 109: 319–322.

O'CONNOR, J. F., AND G. POWER. 1976. Production by brook trout (*Salvelinus fontinalis*) in four streams in the Matamek watershed, Quebec. J. Fish. Res. Board Can. 33: 6–18.

PFEIFFER, R. A. 1955. Studies on the life history of the rosyface shiner *Notropis rubellus*. Copeia 1955: 1083–1088.

PORTT, C. B. 1979. Fish production and the relationship to production and biomass in southern Ontario streams. M.Sc. thesis, University of Guelph, Guelph, Ont. 144 p.

1985. The effects of depth and harvest on baitfish in southern Ontario streams. Ont. Fish. Tech. Rep. Ser. No. 15: iii + 22 p.

PORTT, C. B., AND E. K. BALON. 1984. A potential source of error when estimating ecological production of fishes using back-calculations. Environ. Biol. Fishes 10: 303–305.

RICKER, W. E. 1946. Production and utilization of fish populations. Ecol. Monogr. 16: 373–391.

1975. Computation and interpretation of biological statistics of fish populations. Bull. Fish. Res. Board Can. 191: 382 p.

SCHLOSSER, I. J. 1982. Trophic structure, reproductive success, and growth rate of fishes in a natural and modified headwater stream. Can. J. Fish. Aquat. Sci. 39: 968–978.

SCOTT, W. E., AND E. J. CROSSMAN. 1973. Freshwater fishes of Canada. Bull. Fish. Res. Board Can. 184: 966 p.

SEGERSTRÅLE, C. 1933. Über scalimetrische Methoden zur Bestimmung des linearen Wachstums bei Fischen. Acta Zool. Fenn. 15: 1–168.

SMALL, J. W. JR. 1975. Energy dynamics of benthic fishes in a small Kentucky stream. Ecology 56: 827–840.

STEWART, P. A. M. 1975. Catch selectivity by electrical fishing systems. J. Cons. Int. Explor. Mer 36: 106–109.

SULLIVAN, C. 1956. The importance of size grouping in population estimates employing electric shockers. Prog. Fish-Cult. 18: 188–190.

TRAUTMAN, M. B. 1957. The fishes of Ohio with illustrated keys. Ohio State University Press, Columbus, OH.

WATERS, T. F. 1969. The turnover ratio in production ecology of freshwater invertebrates. Am. Nat. 103: 173–185.

1977. Secondary production in inland waters. Adv. Ecol. Res. 10: 91–164.

WATSON, D. J., AND E. K. BALON. 1984. Structure and production of fish communities in tropical rainforest streams of northern Borneo. Can. J. Zool. 62: 927–940.

WICKLAND, R. E., AND N. R. RICHARDS. 1961. The soil survey of Oxford county. Rep. No. 28, Ontario Soil Survey, Canada Department of Agriculture, Ottawa, Ont.

WILLIAMS, G. C. 1959. Ovary weights of darters: a test of the alleged association of parental care with reduced fecundity in fishes. Copeia 1959: 18–24.

Experimental Analysis of the Direct and Indirect Effects of an Omnivorous Filter-Feeding Clupeid on Plankton Community Structure

Ray W. Drenner

Department of Biology, Texas Christian University, Fort Worth, TX 76129, USA

Stephen T. Threlkeld

Biological Station and Department of Zoology, University of Oklahoma, Kingston, OK 73439, USA

and Michael D. McCracken

Department of Biology, Texas Christian University, Fort Worth, TX 76129, USA

Drenner, R. W., S. T. Threlkeld, and M. D. McCracken. 1986. Experimental analysis of the direct and indirect effects of an omnivorous filter-feeding clupeid on plankton community structure. Can. J. Fish. Aquat. Sci. 43: 1935–1945.

In laboratory trials, feeding rates of an omnivorous filter-feeding clupeid, *Dorosoma cepedianum*, increased as a function of particle size, with maximal rates on microspheres, spherical algae, and zooplankton >40 μm; it did not efficiently feed on filamentous *Anabaena flos-aquae*. To examine the community level impacts of *Dorosoma*, we conducted four seasonal outdoor tank experiments of cross-classified design involving two or three densities of *Dorosoma* and two densities of the zooplanktivorous atherinid fish, *Menidia beryllina*. We attempted to discriminate between the direct and indirect effects of *Dorosoma* on phytoplankton by using *Menidia* to produce indirect effects on phytoplankton by suppressing zooplankton. Experiments began in November, March, June, and September and lasted for 45–53 d. *Dorosoma* suppressed most zooplankton in at least one experiment and enhanced algal standing crops in all four experiments, as indicated by increased algal chlorophyll fluorescence, turbidity, Coulter counts and microscopic algal counts, and decreased Secchi depths. Because in three out of four experiments *Menidia* suppressed zooplankton biomass to a greater extent than *Dorosoma* without enhancing phytoplankton, we reject the hypothesis that the enhancement of phytoplankton by *Dorosoma* was an indirect effect of zooplankton biomass suppression.

En laboratoire, le taux d'alimentation d'un clupéidé filtreur omnivore, *Dorosoma cepedianum*, augmentait en fonction de la taille des particules, avec un maximum pour les microsphères, les algues sphériques et le zooplancton >40 μm; ce poisson ne se nourrissait pas efficacement avec l'algue filamenteuse *Anabaena flos-aquae*. Pour examiner les effets de *Dorosoma* sur la communauté, nous avons effectué quatre expériences saisonnières avec classement à double entrée, dans des bassins placés à l'extérieur, avec deux ou trois densités de *Dorosoma* et deux densités de l'athérinidé zooplanctonivore *Menidia beryllina*. Nous avons essayé de faire la distinction entre les effects directs et indirects de *Dorosoma* sur le phytoplancton en utilisant *Menidia* pour produire des effets indirects sur le phytoplancton en éliminant le zooplancton. Les expériences ont commencé en novembre, mars, juin et septembre et ont duré entre 45 et 53 j. *Dorosoma* a éliminé la plus grande partie du zooplancton dans une expérience au moins et a amélioré la biomasse algale dans toutes les expériences, comme le montrent l'augmentation de la fluorescence chlorophyllienne, la turbidité, les dénombrements au compteur Coulter et au microscope et la diminution de la transparence de l'eau mesurée au disque de Secchi. Étant donné que dans trois des quatre expériences *Menidia* a éliminé la biomasse de zooplancton dans une proportion plus grande que *Dorosoma* sans accroissement du phytoplancton, nous rejetons l'hypothèse selon laquelle l'augmentation du phytoplancton due à *Dorosoma* serait un effet indirect de l'élimination de la biomasse zooplanctonique.

Received March 6, 1986
Accepted June 25, 1986
(J8708)

Reçu le 6 mars 1986
Accepté le 25 juin 1986

Planktivorous fish directly suppress some prey species and also indirectly affect species which interact with these prey (Hrbáček 1962; Brooks and Dodson 1965; Hall et al. 1970). Indirect effects are important because they spread the impacts of a fish beyond its immediate prey to other components of the community. Most studies of planktivore impacts have focused on visually feeding fish that are size-selective zooplanktivores, directly suppressing large zooplankton and indirectly enhancing small zooplankton and phytoplankton (see review by Hurlbert and Mulla 1981). Although filter-feeding fish can dominate fish community biomass of freshwater lakes, their impacts have been less studied (Grygierek 1973; Januszko 1978; Drenner et al. 1984a, 1984b; Threlkeld 1986). Our study is an experimental evaluation of the effects of a filter-feeding clupeid fish, the gizzard shad (*Dorosoma cepedianum*), on plankton community structure. This

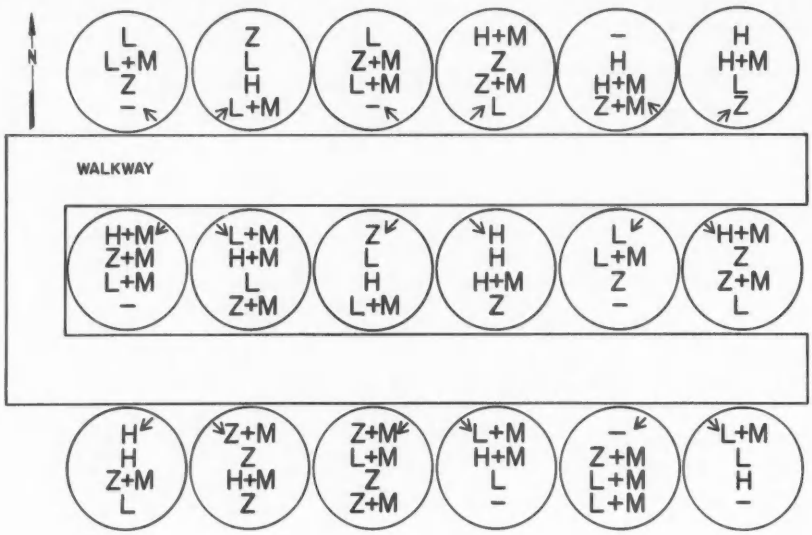


FIG. 1. Layout of the experimental tanks, sampling walkway, and location and direction of outflow from airlift mixers (arrows). Treatment combinations assigned in the four experiments are shown by the column of letters in each tank (Z, L, H = zero, low, and high biomass of *Dorosoma*; M = *Menidia* present); dashes indicate tanks drained for repair in the first experiment or not included in the last experiment. Biomasses of *Dorosoma* or *Menidia* associated with these treatment combinations are listed in Table 1.

species of *Dorosoma* is an omnivore, consuming phytoplankton, zooplankton, and detritus as a pump filter feeder (Kutkuhn 1957; Cramer and Marzolf 1970; Baker and Schmitz 1971; Drenner et al. 1982b). We hypothesized that *Dorosoma* can affect phytoplankton directly through algal grazing or indirectly through effects associated with predation on herbivorous zooplankton.

To evaluate the direct and indirect effects of this omnivore, we conducted four seasonal outdoor tank experiments of cross-classified design involving *Dorosoma* and a zooplanktivorous atherinid fish, the inland silverside (*Menidia beryllina*). We attempted to discriminate between direct and indirect effects of *Dorosoma* by using *Menidia* to produce the indirect effects on phytoplankton caused by suppression of zooplankton by *Dorosoma*. To facilitate interpretation of *Dorosoma*'s direct effects, we conducted laboratory experiments examining the effects of particle type, fish size, and temperature on selective particle ingestion by *Dorosoma*.

Methods

Feeding Experiments

Dorosoma were seined or electrofished from Lake Texoma, Oklahoma–Texas. Fish used in laboratory trials were fed fresh zooplankton and acclimated for at least 1 wk at 15 or 25°C in 180-L plastic pools. Lengths of fish used in the feeding trials are given in the results. Particle consumption was determined by monitoring declines in particle densities in aquaria or pools as two to four *Dorosoma* fed. Particles used in the experiments were polystyrene microspheres (5–52 μm) (Ionics Inc., Watertown, MA), heat-killed zooplankton, and cultured algae. Algae were obtained from W. Carmichael, Wayne State University (toxic strain of *Anabaena flos-aquae*, 10–250 μm long), or purchased from the University of Texas Algal Collection (*Chlorella pyrenoidosas*-UTEX 252, 10 μm in diameter; *Glaucosphaera* sp.-UTEX LB 1661, 18–20 μm in diameter; *Peridinium willei*-UTEX LB 2028, 35 × 40 × 45 μm; *Eremosphaera gigas*-UTEX LB 122, 80–110 μm in diameter; *E. viridis*-UTEX 34, 160–300 μm in diameter). Algae were cultured in 6-L flasks under fluorescent lights using media prescribed by Starr (1978).

Five particle combinations were used in the feeding experiments: (1) microspheres; (2) spherical algae; (3) microspheres and spherical algae; (4) microspheres and *Chlorella*; and (5) microspheres and *Anabaena*. Heat-killed zooplankton were present in all feeding trials so that *Dorosoma* feeding was examined in the context of a complete size array of plankton and because fish would not feed on algae or microspheres alone. Despite stirring of the containers every 10 min, *Eremosphaera* would not stay in suspension and is not included in our analysis of the feeding experiments.

Water samples were taken at 0 and 1 h of feeding by quickly lowering a Plexiglas tube onto a randomly placed rubber stopper on the aquarium or pool bottom. At each time, two samples were taken for analysis of zooplankton, strained through an 80-μm-mesh sieve, and preserved in 5% formalin. Two additional unfiltered samples were taken at both times and preserved in Lugol's solution for analysis of particle size distribution using a model ZM Coulter counter with a 200-μm-aperture tube. *Anabaena* filaments were counted and measured on an inverted microscope at 400×. Zooplankton were counted on a dissecting microscope. Controls for particle loss contained microspheres, algae, and heat-killed zooplankton. Particle loss in the controls averaged 4.6% and was independent of particle size ($F = 0.34$; df = 9,6; $P = 0.54$).

TABLE 1. Living and dead biomass of *Dorosoma* and *Menidia* (grams dry weight) (mean SD)) by experiment and treatment combination. Range of standard lengths of *Dorosoma* (D) and *Menidia* (M) is given for each experiment in the left column. Treatment combination codes Z, L, and H refer to zero, low, and high *Dorosoma* biomass; M refers to *Menidia* present. Refer to Fig. 1 for location in tanks.

Experiment standard lengths(cm)	Treatment combination	Living *Dorosoma* biomass	Dead *Dorosoma* biomass	Living *Menidia* biomass	Dead *Menidia* biomass
Nov.–Dec.	Z	0	0	0	0
D(9–31)	L	119.9(21)	4.4(4)	0	0
M(3–10)	H	251.9(27)	38.2(30)	0	0
	Z+M	0	0	19.1(0)	4.0(0)
	L+M	89.7(34)	−1.5(15)	19.0(2)	4.0(2)
	H+M	271.2(44)	22.7(32)	17.4(1)	5.6(1)
Mar.–Apr.	Z	0	0	0	0
D(7–27)	L	74.6(2)	2.2(2)	0	0
M(5–8)	H	190.7(17)	8.6(8)	0	0
	Z+M	0	0	7.7(0)	6.9(0)
	L+M	61.2(6)	6.9(10)	7.1(1)	7.5(1)
	H+M	173.1(16)	21.9(28)	7.9(1)	6.7(1)
June–July	Z	0	0	0	0
D(9–22)	L	81.2(15)	12.9(8)	0	0
M(4–7)	H	148.8(12)	12.8(23)	0	0
	Z+M	0	0	10.1(3)	7.2(3)
	L+M	78.9(27)	0.2(14)	10.6(1)	6.7(1)
	H+M	124.4(21)	38.2(12)	10.4(0)	6.9(0)
Sept.–Nov.	Z	0	0	0	0
D(10–24)	L	78.7(2)	31.1(1)	0	0
M(6–8)	Z+M	0	0	11.7(1)	5.3(1)
	L+M	83.0(12)	44.6(10)	11.5(2)	5.5(2)

Tank Experiments

Four experiments were conducted in 18 fiberglass tanks located at the University of Oklahoma Biological Station at Lake Texoma. The tanks are 2.3 m in diameter, 2 m high, and hold approximately 7000 L. The tanks are arranged in three rows of six tanks each and are sampled from a walkway between the tanks (Fig. 1). Water was mixed within each tank in the last three experiments by an airlift mixer system. Air was delivered through a polyvinylchloride (PVC) manifold from a Rotron blower and injected at 75 cm depth into a 5-cm-diameter PVC airlift suspended from a surface float. Water was entrained from a depth of 120 cm which was about 50 cm above the bottom of each tank. An average of 85 L water/min passed through the airlift, insuring a well-mixed tank environment.

Experiments began on 5 November 1982 and 11 March, 15 June, and 23 September 1983 and lasted for 45–53 d each under a broad range of temperature conditions (Fig. 2). Changeover periods of 45 d followed each experiment during which additional treatments were imposed on the tank communities. The results of the changeover experiments are not reported here. Sick and dead fish at the surface were removed during daily inspections of the tanks and replaced with live fish from Lake Texoma when available. At the end of each changeover period the tanks were carefully drained through 0.5-cm-mesh seine material and all remaining live fish were recovered. Fish that were unaccounted for at the time of tank draining were assumed to have died and decomposed in the tanks during the experiment. Length measurements of recovered fish were converted to biomass using the length–weight regressions for *Dorosoma* and *Menidia* of J. Korstad and S. T. Threlkeld (unpubl. data). Dead fish biomass was calculated as a time-weighted mean of fish added to the tanks that were not recovered at the end of each experiment. Living fish biomass was calculated as a time-weighted mean of live fish present from daily records of fish removal and replacement and recovery of live fish at the end of each experiment.

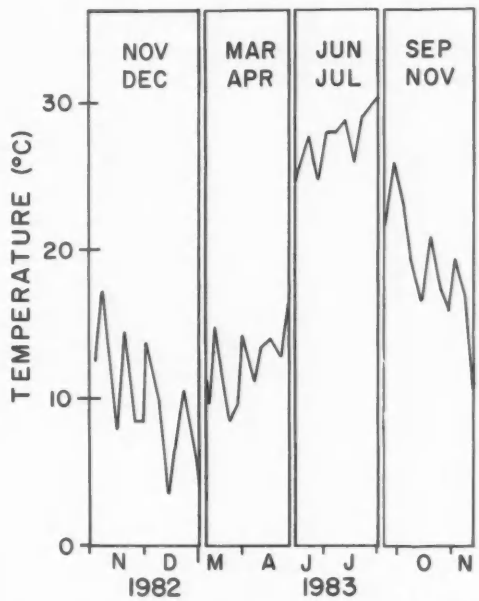


FIG. 2. Mean tank temperatures during the four experiments.

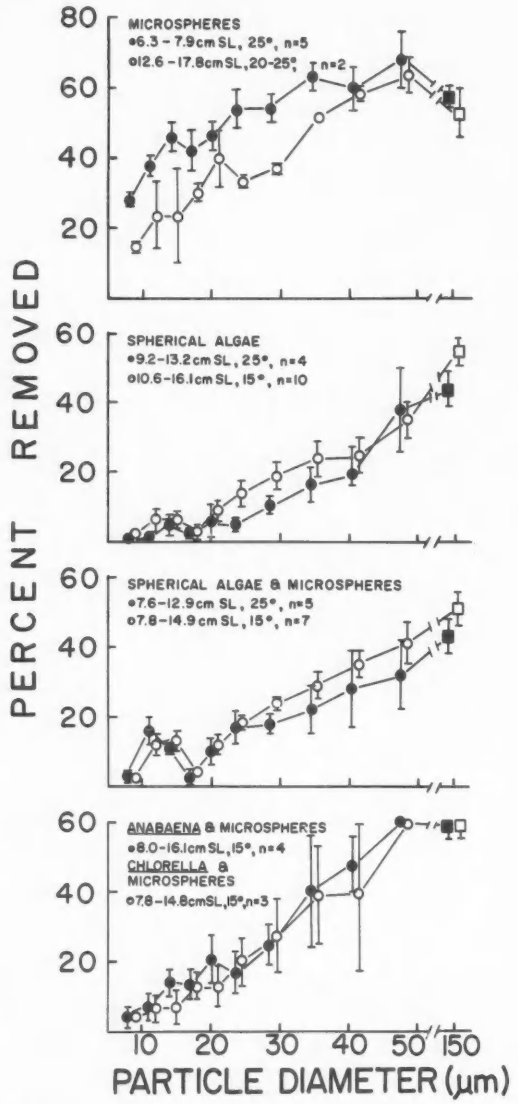


FIG. 3. Percent removal of particles by *Dorosoma* in the feeding trials as a function of particle size (±1 SE). Algae and microspheres are indicated by circles; heat-killed zooplankton, which were present in all trials, are indicated by squares. Each panel presents the results of experiments with a specified particle combination; *Dorosoma* standard lengths, experimental temperatures, and number of experiments are indicated.

The cross-classified design included the effects of *Dorosoma* (three densities except in the last experiment when two densities were used) and *Menidia* (two densities). We used *Menida* because it feeds on zooplankton but not algae (McComas and Drenner 1982) and we hoped that it would mimic only the zooplanktivorous effects of *Dorosoma*; it was also readily available in Lake Texoma. The cross-classified design was chosen because it allows simultaneous evaluation of the main effects of the two fish species and their interaction. Comparison of main effects of the two fish species provides the basis for evaluating the direct and indirect effects due to omnivory of *Dorosoma* versus the zooplanktivory of *Menidia*. Interaction terms allow assessment of whether *Dorosoma* effects on phytoplankton were independent of variation in zooplankton as provided by the *Menidia* treatment.

Figure 1 shows the distribution of treatment combinations to the tanks. Lengths of fish and the biomass of living and dead fish for the treatment combinations are given in Table 1. All treatment combinations were replicated threefold, except in the first experiment when two tanks were drained for repair. ANOVA of pretreatment measurements of algal chlorophyll fluorescence, turbidity, and Secchi depths did not reveal any treatment effects due to tank location or pretreatment variation. We found a significant *Menidia* effect on tank temperature ($P = 0.027$) in the first experiment, apparently because half of the tanks with *Menidia* had southern exposure. In the other experiments, treatment assignments were distributed with the restriction that a treatment combination could not be repeated in any row or in any east–west block of six tanks. Insulation was added to the tanks after the first experiment.

Prior to each experiment, the tanks were drained, scrubbed, and particulate material removed by vacuum. Each experiment was initiated by filling the tanks with water from Lake Texoma delivered through a 5-cm PVC pipe by a centrifugal pump with intake 65 m offshore in Lake Texoma. All tanks were filled in 18–24 h. In addition to the natural complement of zooplankton and phytoplankton present in the water pumped into the tanks, plankton were collected from the lake in water of 15 m depth with 15–18 vertical tows of a 64-μm-mesh net, mixed together, and distributed in equal aliquots to each of the tanks at the beginning of an experiment. Plankton species composition and density in the tanks were similar to those found in the lake (S. T. Threlkeld, unpubl. data). Because pilot experiments showed that sand is important to digestion of phytoplankton by *Dorosoma*, we added 4 L of lakeshore sand to each tank at the beginning of the experiment.

Tanks were usually monitored at 5-d intervals. Zooplankton were sampled with vertical tows of an 80-μm-mesh Wisconsin plankton net, one tow per tank every other sampling date (five dates per experiment). Samples were preserved in 5% sucrose formalin and counted under a dissecting microscope.

Phytoplankton samples were collected at the beginning, middle, and end of each experiment with a 2.1-m-long, 1.5-cm-diameter PVC tube lowered to near the tank bottom and then closed from the top. Samples were preserved with 0.5% Lugol's iodine. Phytoplankton samples were settled in 10-mL chambers for 24 h. Eight fields were counted on an inverted microscope at 400×. Ciliated protozoans were rarely encountered in these samples and were never abundant. Periphyton was noticeable on the walls of the tanks late in the experiments but was not measured.

Samples for Coulter counting and water quality analysis were also taken with the PVC tube (nine dates per experiment). Coulter counter samples were preserved in 10% formalin. Particles ranging from 5 to 60 μm were counted with a model ZM Coulter counter with a 200-μm-aperture tube. Turbidity was measured with a model 2100A Hach turbidimeter. Algal chlorophyll fluorescence was measured in vivo with an Aminco fluorometer. Fluorescence was also determined after addition of DCMU solution (final concentration of 10^{-5} *M*; Vincent 1981).

Water samples for the determination of ammonia, nitrate, and soluble reactive phosphorus were collected on four dates in

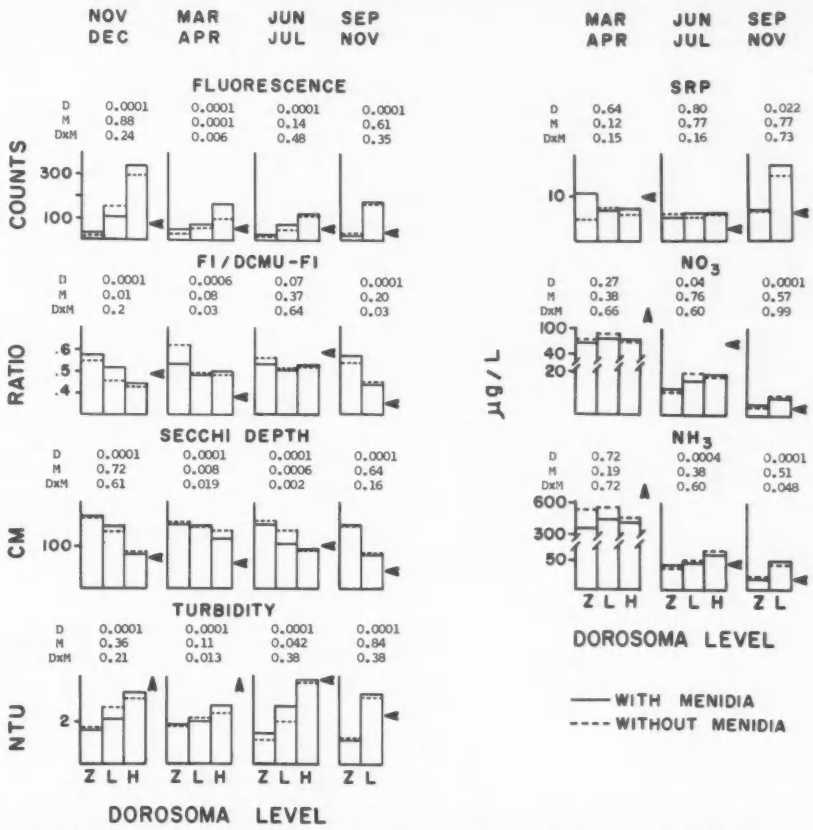


FIG. 4. Mean responses of water quality and nutrient parameters to *Dorosoma* and *Menidia* treatments. *Dorosoma* level (Z, L, H) refers to zero, low, and high biomass (see Table 1 for details). Probabilities from repeated measures ANOVA for main effects of *Dorosoma* (D) and *Menidia* (M) or an interaction between *Dorosoma* and *Menidia* (D×M) are indicated above the histogram for each parameter and experiment. Left-pointing arrows to the right of each histogram indicate the initial value of the parameter in the experiment. Off-scale starting values are indicated by upward-pointing arrows.

each of the last three experiments, filtered through Gelman HA (0.45 µm) glass fiber filters, and analyzed using salicylate (Verdouw et al. 1978), cadmium reduction, and molybdate methods, respectively (American Public Health Association et al. 1980). Alkalinity was determined by potentiometric methods (American Public Health Association et al. 1980).

Analysis of the resulting data sets was done primarily by ANOVA for factors with repeated measurement, modified in the November–December experiment for unequal replication (Winer 1971; Gill 1978). In ANOVA for factors with repeated measurements, the error term for evaluation of main effects and their interactions is based on replication of experimental units (usually three tanks per treatment combination). Replication within units (dates within an experiment) defines the error term used to evaluate temporal effects and interactions of the treatment factors (*Dorosoma* or *Menidia*) with time. Because tank communities were uniform at the start of each experiment, temporal effects and treatment × time interactions were not fundamentally different from those revealed by analysis for main effects and interactions. Initial conditions for most parameters were usually intermediate in the range of observed experimental responses, indicating that the main temporal effect of *Dorosoma* or *Menidia* treatments was divergence. Although a double blocking system was used for treatment assignment in the last three experiments, no significant block effects were detected (all $P > 0.1$) and block-related terms were pooled with the error terms. Given the replication involved in the experiments, a deviation of 1, 2, and 3 $\sqrt{MS_e}$ could be detected as significantly different at $\alpha = 0.05$ with probabilities of 0.26, 0.76, and 0.98 (experimental power), respectively. Multiple regression analysis of the 64 tank-specific means of each variable measured in the four experiments (16, 18, 18, and 12 tanks, respectively) was done against the treatment factors and nuisance effects associated with fish mortality.

Results

Feeding Experiments

In all particle combinations, *Dorosoma* feeding rates increased as a function of particle size, with highest feeding rates on particles and zooplankton >40 µm (Fig. 3). Multiple regression of all variables included in the experiments showed that particle selectivity (measured as relative percent removal rates) significantly increased with particle size and decreased with fish size and in the presence of algae (all $P < 0.05$), but

TABLE 2. Multiple regression of response variables on living and dead *Dorosoma* and *Menidia* biomass (64 tank-specific means; df = 59). Partial r^2 values (where $P < 0.05$) are given and preceded by sign of regression coefficient.

Parameter	Explained variation (%)	*Dorosoma* weight	*Dorosoma* loss	*Menidia* weight	*Menidia* loss
Fluorescence	79	+0.62	+0.30	+0.12	
F1/DCMU-fluorescence	49	−0.29	−0.11		
Secchi depth	64	−0.31	−0.34		
Turbidity	62	+0.38	+0.22		
Alkalinity	14				
Ammonia	23		−0.08	−0.12	+0.11
Nitrate	28	+0.11	−0.08	−0.15	+0.15
Phosphorus	29		+0.20	+0.09	−0.09
Algal cells <2 μm	51	+0.31	+0.12		
Blue-green unicells	19		+0.18		
Cryptophytes	24	+0.19			
Blue-green colonies	22		+0.21		
Green unicells	39	+0.29			
Green colonials	25		+0.21		
Blue-green filaments	18				
Centric diatoms	36		+0.28	+0.14	−0.12
Pennate diatoms	13		+0.10		
Chrysophytes	10				
5−10 μm particles	65	+0.23	+0.40		
10−15 μm particles	57	+0.14	+0.35		
15−20 μm particles	48	+0.17	+0.21		
20−25 μm particles	43	+0.21			+0.06
25−30 μm particles	27	+0.14			
30−35 μm particles	24	+0.13			
Brachionus	8				+0.07
Keratella	36			−0.14	+0.33
Bosmina	16				
Ceriodaphnia	20				
Daphnia	32	−0.13			−0.08
Diaphanosoma	12				
Nauplii	22			−0.19	
Cyclopoid copepodids	29	−0.09		−0.11	
Diaptomus copepodids	23			−0.08	
Eurytemora copepodids	20			−0.08	

was independent of temperature ($P > 0.05$). *Dorosoma* did not reduce the abundance ($F = 0.17$; df = 1,48; $P = 0.89$) or change the size frequency distribution ($F = 0.73$; df = 11,48; $P = 0.71$) of *Anabaena* filaments.

Tank Experiments

Dorosoma enhanced algal standing crops in the four experiments, as indicated by increased algal fluorescence, turbidity, and Coulter counts, and decreased Secchi depths (Fig. 4 and 5). All algal taxa were enhanced by *Dorosoma* in at least one experiment (Fig. 6). Only one algal group, colonial blue-greens in the March−April experiment, was suppressed by *Dorosoma*. The ratio of fluorescence to DCMU-enhanced fluorescence was always lower in the presence of *Dorosoma* (Fig. 4), indicating better algal health (Samuelsson et al. 1978; Vincent 1981).

Zooplankton biomass, composed primarily of cladocerans, was suppressed by *Dorosoma* in three experiments (Fig. 7). Most zooplankton taxa were suppressed by *Dorosoma* in at least one experiment (Fig. 8). Blue-green colonies, *Diaptomus*, and *Polyarthra* were suppressed in one experiment and enhanced in another. *Dorosoma* increased soluble reactive phosphorus in the September−November experiment and ammonia and nitrate levels in the June−July and September−November experiments (Fig. 4).

Menidia enhanced algal standing crops only in the March−April experiment, as indicated by algal fluorescence and Coulter counts (Fig. 4 and 5). Most algal taxa were not affected by *Menidia* (Fig. 6), although green colonies, pennate diatoms, and blue-green filaments were all significantly enhanced in at least one experiment. *Menidia* suppressed total zooplankton biomass, cladocerans, and copepods in all experiments and enhanced rotifers except in the September−November experiment when only *Brachionus* was present (Fig. 7 and 8). We detected no effects of *Menidia* on nutrients (Fig. 4).

Significant interactions between *Dorosoma* and *Menidia* were observed for most zooplankton taxa and biomass in at least one experiment (Fig. 7 and 8). In some cases, the effects of one fish offset the effects of the other species (compensatory, e.g. *K. cochlearis*) but in most cases the effects of the two fish were similar (synergistic, e.g. *Daphnia*, zooplankton biomass). Synergistic interactions between *Dorosoma* and *Menidia* on phytoplankton were observed in the March−April experiment, as indicated by algal fluorescence and Coulter counts. *Dorosoma*−*Menidia* synergistic interactions were de-

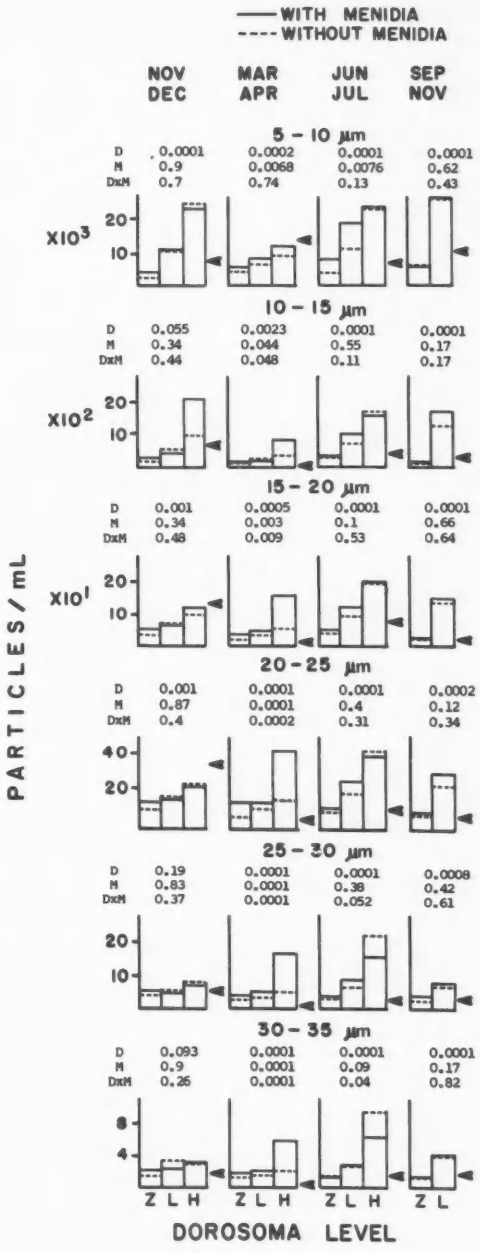


FIG. 5. Mean responses of particle density as measured by Coulter counter to *Dorosoma* and *Menidia* treatments. *Dorosoma* level (Z, L, H) refers to zero, low, and high biomass (see Table 1 for details). Probabilities from repeated measures ANOVA for main effects of *Dorosoma* (D) and *Menidia* (M) or an interaction between *Dorosoma* and *Menidia* (D×M) are indicated above the histogram for each parameter and experiment. No significant effects were observed for particles >35 µm which were rare and are not presented. Left-pointing arrows to the right of each histogram indicate the initial value of the parameter in the experiment. Off-scale starting values are indicated by upward-pointing arrows.

tected in one experiment each for green unicells, green colonies, and blue-green filaments.

To evaluate the effects of fish mortality in the context of treatment effects, the 64 tank-specific means of each response variable measured in the four experiments (16, 18, 18, and 12 tanks, respectively) were regressed against the major treatment variables (live biomass of *Dorosoma* and *Menidia*) and the nuisance variables associated with mortality of introduced *Dorosoma* and *Menidia* (dead fish biomass). Because live and dead fish biomass were poorly correlated ($r = 0.43$), these two variables were treated as independent predictor variables in the multiple regression analysis. Many *Dorosoma* effects on water quality and algal parameters could be attributed to both living and dead fish biomass (at $P < 0.05$), but few significant effects could be attributed to *Menidia* (Table 2). *Menidia* effects on zooplankton were consistent with the results of ANOVA of individual experiments.

Discussion

Because of food web complexity, it is often difficult to distinguish between direct and indirect community effects of fish, even within the context of field experiments. The fish-free controls that are typically employed to evaluate the net effect of fish on a community do not necessarily allow assessment of the underlying mechanisms. Mechanistic interpretations usually depend on corroborative data rather than a test specified by the experimental design. In the tank experiments, we attempted to determine how *Dorosoma* affects phytoplankton by using *Menidia* as a control for indirect effects resulting from suppression of zooplankton.

Plankton suppression is often interpreted as a direct effect of fish feeding, especially when the observed responses of field populations are consistent with corroborative laboratory feeding trials or analyses of fish stomach contents. Many of the *Menidia* or *Dorosoma* effects that we observed were consistent with this view of fish impacts on community structure. *Menidia* suppressed all cladoceran and copepod species, the largest bodied zooplankton in the tank communities. While there are few studies of atherinid impacts on plankton communities (Zaret and Kerfoot 1975; Threlkeld 1986), suppression of large-bodied zooplankton is typical of visual feeders (Hurlbert and Mulla 1981). Laboratory studies show that *Menidia* is a size-selective zooplankton predator when feeding in the light but in total darkness will consume zooplankton as a filter feeder (McComas and Drenner 1982). Compared with its impact as a visual feeder, its impact as a filter feeder is negligible because the volume cleared of prey when filter feeding is much smaller than the volume that can be searched and cleared of prey when visually feeding.

Although our feeding trials show *Dorosoma* to feed as an omnivore, the primary suppression of plankton by *Dorosoma* was limited to most cladocerans, and some copepod and rotifer species. *Dorosoma* does not have the same effects on zooplankton as *Menidia* because they have different feeding behaviors. *Dorosoma* does not visually locate individual prey like *Menidia* but instead filter feeds using a rapid series of suctions to draw prey into its buccal cavity where they are strained by the gill rakers (Drenner et al. 1978). Our experiments show that small *Dorosoma* attain near-maximal feeding rates at a smaller particle size than do larger *Dorosoma*. This shift in feeding efficiency is consistent with changes in gill raker spaces with fish size (Mummert and Drenner 1986).

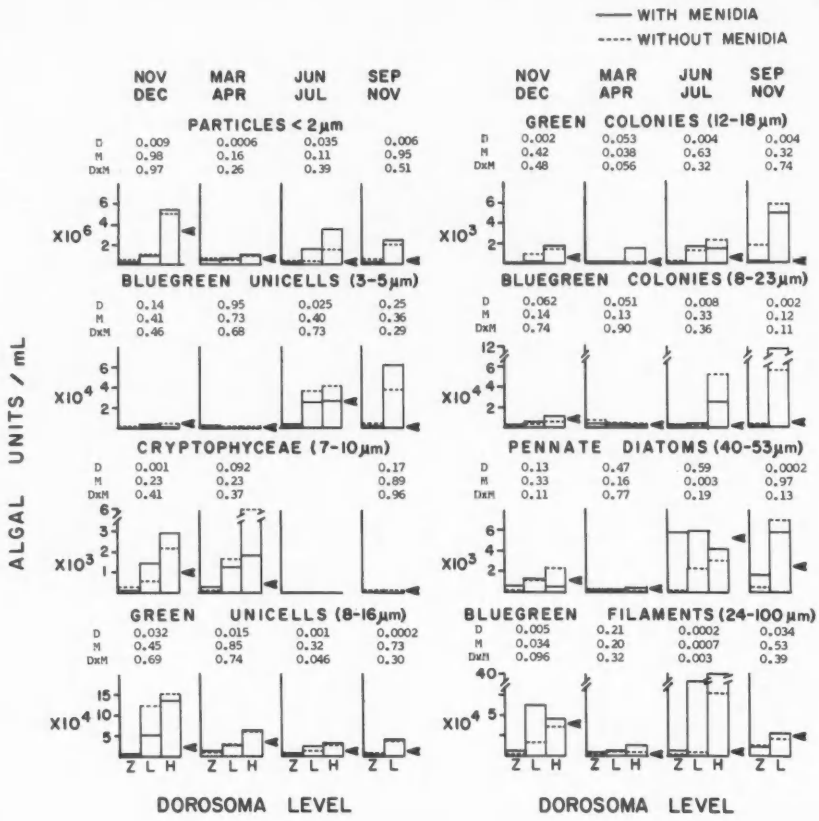


FIG. 6. Mean responses of algal taxa to *Dorosoma* and *Menidia* treatments. *Dorosoma* level (Z, L, H) refers to zero, low, and high biomass (see Table 1 for details). Probabilities from repeated measures ANOVA for main effects of *Dorosoma* (D) and *Menidia* (M) and interaction between *Dorosoma* and *Menidia* (D×M) are indicated above each histogram for each parameter and experiment. Algal sizes are also given. Left-pointing arrows to the right of each histogram indicate the initial value of the parameter in the experiment. Off-scale starting values are indicated by upward-pointing arrows.

Filtering efficiency is not the only factor controlling selective feeding by *Dorosoma*. *Dorosoma* has low feeding rates for prey such as calanoid copepods which can evade the fish's suction (Drenner et al. 1978). *Dorosoma* enhanced *Diaptomus* copepods in the June–July tank experiment, although it suppressed other evasive forms in other experiments (cyclopoids and *Diaptomus* in March–April), suggesting strong seasonal aspects of these interactions. Enhancement of *Diaptomus* by *Dorosoma* during the summer was also observed by Drenner et al. (1982a) in a pond experiment.

Most of the phytoplankton in the tank communities were smaller than 40 μm and would experience less than maximal grazing pressure by *Dorosoma*. Even blue-green filaments (10–250 μm) and pennate diatoms (40–53 μm) may not be efficiently grazed. Elongated particles are not filtered by a sieve according to their longest axial dimension (Runge and Ohman 1982). *Dorosoma* did not graze *Anabaena* in the feeding trials, and Mummert and Drenner (1986) found that the plate-shaped alga *Pediastrum* (93 μm in diameter) is grazed at a rate equivalent to those of spherical particles of 20–26 μm. In addition to low grazing rates, most algal taxa in our study have been found to be able to remain viable after passage through *Dorosoma*'s digestive tract (Velasquez 1939; Smith 1963). Algal populations may also be more able than zooplankton to compensate for grazing mortality because of higher population turnover rates (Bottrell et al. 1976; Reynolds 1984).

The enhancement of phytoplankton by planktivorous fish has been assumed to be an indirect effect of suppression of zooplankton grazing (review by Threlkeld 1986). We attempted to test this hypothesis by using *Menidia* to manipulate the density of zooplankton independently of *Dorosoma*. *Menidia* did not control for all of *Dorosoma*'s indirect effects because *Menidia*'s impact on some zooplankton species differed from *Dorosoma*'s. One difference was that *Menidia* enhanced some rotifers that were suppressed by *Dorosoma*. But in terms of zooplankton biomass, the enhancement of rotifers by *Menidia* was more than offset by the more effective suppression of crustaceans by *Menidia* as compared with *Dorosoma*. We conclude that our use of *Menidia* as a control for the indirect effects of *Dorosoma*'s zooplanktivory erred on the conservative side and is valid. We feel that it is unlikely that differences in zooplankton species composition between *Menidia* and

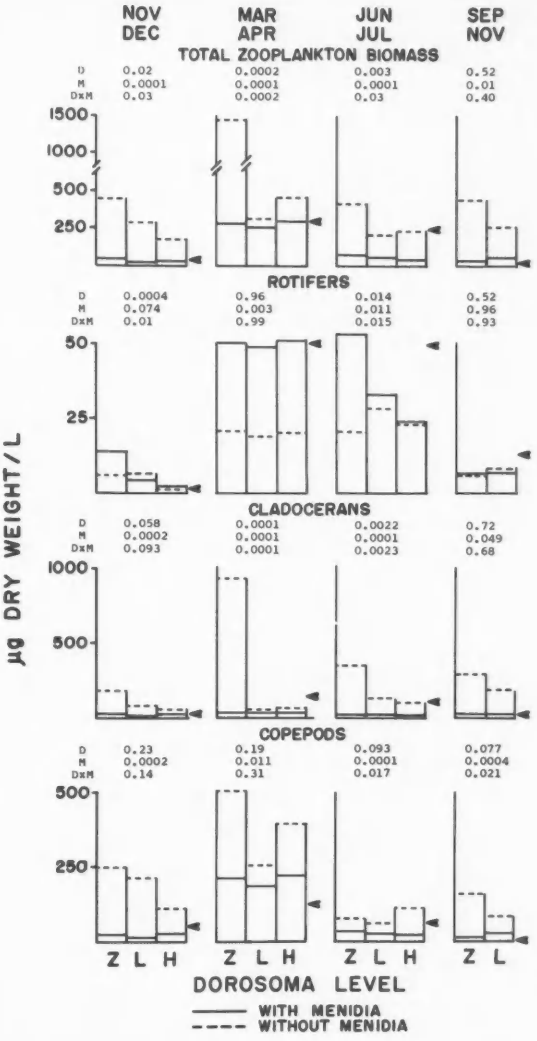


FIG. 7. Mean responses of zooplankton biomass to *Dorosoma* and *Menidia* treatments. *Dorosoma* level (Z, L, H) refers to zero, low, and high biomass (see Table 1 for details). Probabilities from repeated measures ANOVA for main effects of *Dorosoma* (D) and *Menidia* (M) and interactions between *Dorosoma* and *Menidia* (D×M) are given above each histogram. Left-pointing arrows to the right of each histogram indicate the initial value of the parameter in the experiment. Off-scale starting values are indicated by upward-pointing arrows.

Dorosoma treatments could account for the large differences in phytoplankton communities that were observed between these treatments.

In the November–December, June–July, and September–November experiments, *Menidia* suppressed zooplankton biomass to a greater extent than *Dorosoma* but without an enhancement of phytoplankton, permitting us to reject the hypothesis that the enhancement of phytoplankton by *Dorosoma* was an indirect effect of zooplankton biomass suppression. This finding is supported by the lack of significant interactions between *Menidia* and *Dorosoma* on algal chlorophyll fluorescence which shows that the effects of *Dorosoma* on phytoplankton were independent of *Menidia*'s effects (i.e. suppression of zooplankton). We cannot reject this hypothesis for the March–April experiment because the suppression of zooplankton biomass by *Menidia* was accompanied by phytoplankton enhancement. In this experiment, *Dorosoma* and *Menidia* did have a significant interaction of algal fluorescence showing that algal enhancement by *Dorosoma* was not independent of zooplankton suppression by *Menidia*.

An alternative hypothesis is that *Dorosoma* enhanced phytoplankton by enhancing nutrient supply by nutrient regeneration of living fish (Lamarra 1975) or by decomposition of fish that died during the experiments (Kitchell et al. 1975; Durbin et al. 1979). Although no controls for nutrient effects were included in the experimental design, there is some evidence that nutrients played a major role in phytoplankton enhancement. Improved algal condition in the presence of *Dorosoma*, indicated by lower ratios of fluorescence to DCMU-enhanced fluorescence, was consistent with this hypothesis. We also detected increases in the concentration of phosphorus and nitrogen with *Dorosoma* in two of the experiments. Because *Dorosoma* and *Menidia* have approximately equal weight-specific phosphorus excretion rates (J. Korstad and S. T. Threlkeld, unpubl. data), the higher biomass of *Dorosoma* would have increased these nutrient supply effects. Threlkeld (1986) showed that phytoplankton community responses were indistinguishable when *Menidia* and *Dorosoma* were stocked at lower, but equal biomasses, even though their effects on zooplankton differed.

Fish mortality and decomposition may have also contributed to phytoplankton enhancement. Regression of the 64 tank-specific means showed that algal chlorophyll fluorescence was significantly related to the biomass of living *Dorosoma* (partial $r^2 = 0.64$) and to the weight of decomposed *Dorosoma* (partial $r^2 = 0.30$). Secchi depth and turbidity were also significantly related to both living and lost biomass, while most of the algal taxa and Coulter counter size categories were significantly affected by either living or lost *Dorosoma* biomass (Table 2). Similar effects of *Menidia* on the phytoplankton were not detected by the multiple regression analyses. Experimental manipulation of dead fish biomass in subsequent experiments (Threlkeld 1986) confirmed the importance of this source of nutrients to phytoplankton enhancement and showed its importance to be independent of zooplankton abundance. These results suggest that in lakes where *Dorosoma* populations suffer winterkills (Adams et al. 1985), *Dorosoma* mortality and decomposition may contribute to phytoplankton enhancement.

Based on an observation that phytoplankton dominated the diet of young-of-the-year *Dorosoma* and that strong young-of-the-year classes of *Dorosoma* were associated with decreases in summer populations of blue-green algae, Kutkuhn (1957) hypothesized that *Dorosoma* might suppress populations of objectionable algae. Drenner et al. (1984a) confirmed that large *Dorosoma* could reduce algal biomass due to its grazing of the large dinoflagellate *Ceratium*, but our experiments suggest that large *Dorosoma* are not effective controls of algal abundance when the community is dominated by filamentous blue-green algae or nannoplankton. Because algal mortality due to the low grazing rates of large *Dorosoma* is offset by indirect enhancement effects, any short-term suppression of algae by young-of-the-year *Dorosoma* must be weighed against the enhancement of algae by large *Dorosoma*.

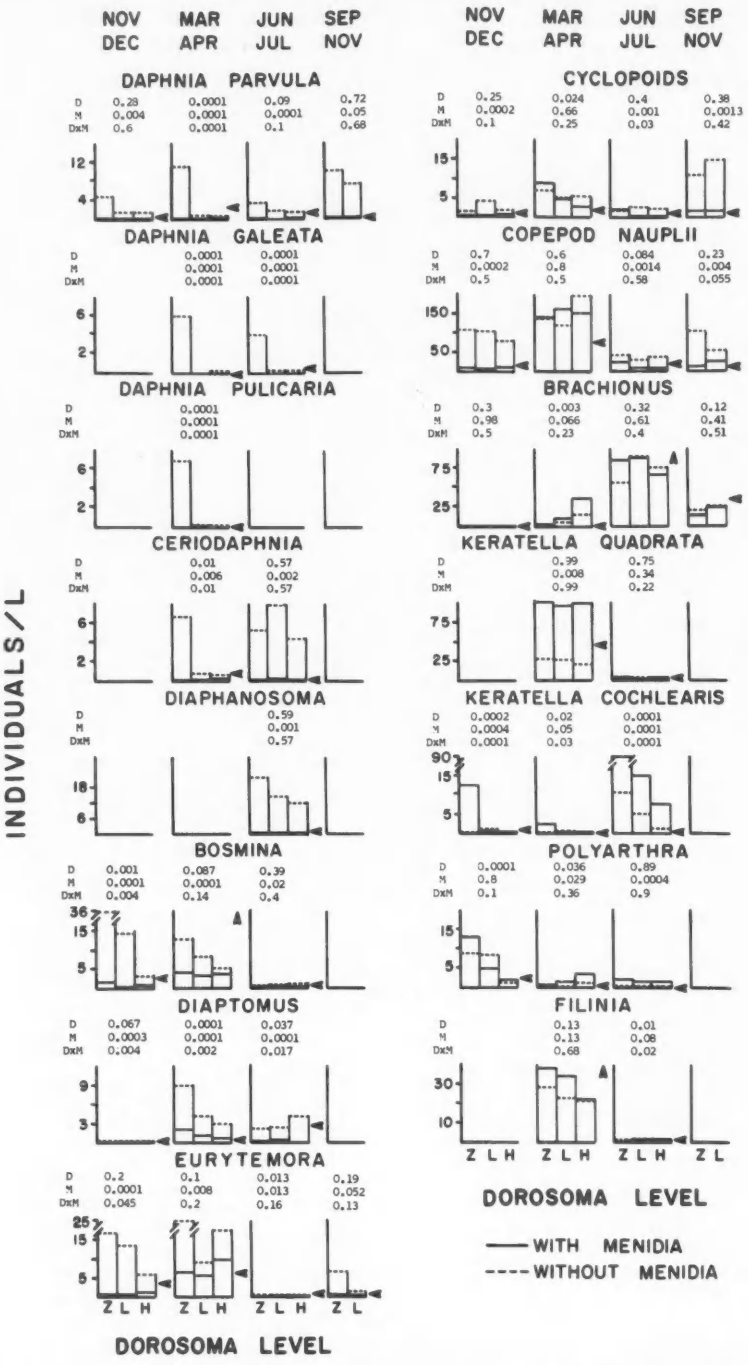


FIG. 8. Mean responses of zooplankton taxa to *Dorosoma* and *Menidia* treatments. *Dorosoma* level (Z, L, H) refers to zero, low, and high biomass (see Table 1 for details). Probabilities from repeated measures ANOVA for main effects of *Dorosoma* (D) and *Menidia* (M) and interactions between *Dorosoma* and *Menidia* (D×M) are given above each histogram. Left-pointing arrows to the right of each histogram indicate the initial value of the parameter in the experiment. Off-scale starting values are indicated by upward-pointing arrows.

Acknowledgements

We are especially grateful to E. Choinski, J. Mummert, and S. Wiseman for their assistance in conducting the experiments. We also appreciate the assistance provided by W. Carmichael, J. Korstad, C. Johnson, E. Wallace, W. Sims, B. Hysmith, and the staff of the Texas Department of Parks and Wildlife at Lake Texoma. We thank the U.S. Environmental Protection Agency for use of their mobile laboratory. Ten of the tanks were purchased by the University of Oklahoma Graduate College. The research was supported by National Science Foundation DEB 8206850 and 8206865.

References

ADAMS, S. M., J. E. BRECK, AND R. B. MCLEAN. 1985. Cumulative stress-induced mortality of gizzard shad in a southeastern U.S. reservoir. Environ. Biol. Fishes 13: 103–112.

AMERICAN PUBLIC HEALTH ASSOCIATION ET AL. 1980. Standard methods for the examination of water and wastewater. 15th ed. American Public Health Association, Washington, DC.

BAKER, C. D., AND E. H. SCHMITZ. 1971. Food habits of adult gizzard and threadfin shad in two Ozark reservoirs, p. 3–11. *In* Gordon E. Hall [ed.] Reservoir fisheries and limnology. Am. Fish. Soc. Spec. Publ. No. 8.

BOTTRELL, H. H., A. DUNCAN, Z. M. GLIWICZ, E. GRYGIEREK, A. HERZIG, A. HILLBRICHT-ILKOWSKA, H. KURASAWA, P. LARSSON, AND T. WEGLENSKA. 1976. A review of some problems in zooplankton production studies. Norw. J. Zool. 24: 419–456.

BROOKS, J. L., AND S. I. DODSON. 1965. Predation, body size, and composition of plankton. Science (Wash., DC) 150: 28–35.

CRAMER, J. D., AND G. R. MARZOLF. 1970. Selective predation on zooplankton by gizzard shad. Trans. Am. Fish. Soc. 99: 320–332.

DRENNER, R. W., J. R. STRICKLER, AND W. J. O'BRIEN. 1978. Capture probability: the role of zooplankter escape in the selective feeding of planktivorous fish. J. Fish. Res. Board Can. 35: 1370–1373.

DRENNER, R. W., F. DENOYELLES, JR., AND D. KETTLE. 1982a. Selective impact of filter-feeding gizzard shad on zooplankton community structure. Limnol. Oceanogr. 27: 965–968.

DRENNER, R. W., W. J. O'BRIEN, AND J. R. MUMMERT. 1982b. Filter-feeding rates of gizzard shad. Trans. Am. Fish. Soc. 111: 210–215.

DRENNER, R. W., J. R. MUMMERT, F. DENOYELLES, JR., AND D. KETTLE. 1984a. Selective particle ingestion by a filter-feeding fish and its impact on phytoplankton community structure. Limnol. Oceanogr. 29: 941–948.

DRENNER, R. W., S. B. TAYLOR, X. LAZZARO, AND D. KETTLE. 1984b. Particle-grazing and plankton community impact of an omnivorous cichlid. Trans. Am. Fish. Soc. 113: 397–402.

DURBIN, A. G., S. W. NIXON, AND C. A. OVIATT. 1979. Effects of the spawning migration of the alewife, *Alosa pseudoharengus*, on freshwater ecosystems. Ecology 60: 8–17.

GILL, J. L. 1978. Design and analysis of experiments in the animal and medical sciences. Vol. 1–3. The Iowa State University Press, Ames, IA.

GRYGIEREK, E. 1973. The influence of phytophagous fish on pond zooplankton. Aquaculture 2: 197–208.

HALL, D. J., W. E. COOPER, AND E. E. WERNER. 1970. An experimental approach to the production dynamics and structure of freshwater animal communities. Limnol. Oceanogr. 15: 839–928.

HRBÁČEK, J. 1962. Species composition and the amount of zooplankton in relation to the fish stock. Rozpr. Cesk. Akad. Ved Rada Mat. Prir. Ved 72(10): 1–116.

HURLBERT, S. H., AND M. S. MULLA. 1981. Impacts of mosquitofish (*Gambusia affinis*) predation on plankton communities. Hydrobiologia 83: 125–151.

JANUSZKO, M. 1978. The influence of silver carp (*Hypophthalmichthys molitrix* Val.) on eutrophication of the environment of carp ponds. Part III. Phytoplankton. Rocz. Nauk Roln. Ser. H Rybactwo 99 (2): 55–79.

KITCHELL, J. F., J. F. KOONCE, AND P. S. TENNIS. 1975. Phosphorus flux through fishes. Verh. Int. Ver. Limnol. 19: 2478–2484.

KUTKUHN, J. H. 1957. Utilization of plankton by juvenile gizzard shad in a shallow prairie lake. Trans. Am. Fish. Soc. 87: 80–103.

LAMARRA, V. A. JR. 1975. Digestive activities of carp as a major contributor to the nutrient loading of lakes. Verh. Int. Ver. Limnol. 19: 2461–2468.

MCCOMAS, S. R., AND R. W. DRENNER. 1982. Species replacement in a reservoir fish community: silverside feeding mechanics and competition. Can. J. Fish. Aquat. Sci. 39: 815–821.

MUMMERT, J. R., AND R. W. DRENNER. 1986. Effect of fish size on the filtering efficiency and selective particle ingestion of a filter-feeding clupeid. Trans. Am. Fish. Soc. 115. (In press)

REYNOLDS, C. S. 1984. The ecology of freshwater phytoplankton. Cambridge University Press, New York, NY.

RUNGE, J. A., AND M. D. OHMAN. 1982. Size fractionation of phytoplankton as an estimate of food available to herbivores. Limnol. Oceanogr. 27: 570–576.

SAMUELSSON, G., G. OQUIST, AND P. HALLDAL. 1978. The variable chlorophyll a fluorescence as a measure of photosynthetic capacity in algae. Mitt. Int. Ver. Limnol. 21: 207–215.

SMITH, W. L. 1963. Viable algal cells from the gut of the gizzard shad *Dorosoma cepedianum* (LeSueur). Proc. Okla. Acad. Sci. 43: 148–149.

STARR, R. C. 1978. The culture collection of algae at the University of Texas at Austin. J. Phycol. 14 (Suppl.): 47–100.

THRELKELD, S. T. 1986. Experimental evaluation of trophic-cascade and nutrient-mediated effects of planktivorous fish on plankton community structure. *In* W. C. Kerfoot and A. Sih [ed.] Predation: direct and indirect impacts on aquatic communities. University Press of New England, Hanover, NH. (In press)

VELASQUEZ, G. T. 1939. On the viability of algae obtained from the digestive tract of the gizzard shad, *Dorosoma cepedianum* (LeSueur). Am. Midl. Nat. 22: 376–412.

VERDOUW, H., C. J. A. VAN ECHTELD, AND E. M. J. DEKKERS. 1978. Ammonia determination based on indophenol formation with sodium salicylate. Water Res. 12: 399–402.

VINCENT, W. F. 1981. Photosynthetic capacity measured by DCMU-induced chlorophyll fluorescence in an oligotrophic lake. Freshwater Biol. 11: 61–78.

WINER, B. J. 1971. Statistical principles in experimental design. 2nd ed. McGraw-Hill, New York, NY.

ZARET, T. M., AND W. C. KERFOOT. 1975. Fish predation on Bosmina longirostris: body-size selection versus visibility selection. Ecology 56: 232–237.

Selection for Adult Size in Female Coho Salmon (*Oncorhynchus kisutch*)

L. B. Holtby and M. C. Healey

Department of Fisheries and Oceans, Fisheries Research Branch, Pacific Biological Station, Nanaimo, B.C. V9R 5K6

Holtby, L. B., and M. C. Healey. 1986. Selection for adult size in female coho salmon (*Oncorhynchus kisutch*). Can. J. Fish. Aquat. Sci. 43: 1946–1959.

Several recent studies have presented evidence that large size confers a selective advantage to female Pacific salmon. Nevertheless, a wide range of female sizes is normally present in any spawning population. Two possible explanations exist for the observed range in female size. First, average female size might be determined by an optimizing process with variation around the optimum size due to individual differences in success at obtaining food. Second, various sizes of females might coexist as a mixed evolutionary stable strategy. Under the first explanation, females of sizes other than the optimum would display lower fitness whereas, under the second explanation, females of all sizes would be equally fit. We investigated factors affecting survival of eggs, fry, and smolts of coho salmon (*Oncorhynchus kisutch*) in Carnation Creek on Vancouver Island with a view to determining the relative fitness of different sized females. Egg-to-fry mortality was best explained by a model that included only the effects of stream bed scour and gravel quality. Including an effect of female size, expressed through depth of egg burying, worsened the model's predictive capability. We could find no evidence that the eggs of large females consistently survived better during incubation than those of small females. In fact, we observed three instances in which it appeared that the eggs of small females survived better. In Carnation Creek, large 1- and 2-yr-old smolts did not consistently survive better in the marine environment than small smolts. Thus, we were unable to demonstrate that the reproductive success of large females was consistently higher than that of small females, contrary to the hypothesis that female size is the result of an optimizing process. In Carnation Creek the observed range of female sizes probably represents an evolutionary stable strategy in which all sizes have equal fitness. We propose a model that predicts female size and variance in size based on the conflicting selective effects of gravel quality, scour, and competition for nest sites.

Plusieurs études récentes présentent des données montrant que le fait d'être de grande taille donne un avantage sélectif aux femelles du saumon du Pacifique. Toutefois, on observe normalement dans toute population de géniteurs une vaste gamme de taille des femelles. On peut donner à ce phénomène deux explications. Premièrement, la taille moyenne des femelles pourrait être déterminée par un processus d'optimisation, avec des variations autour de la taille optimale à cause des différences individuelles dans l'obtention de la nourriture. Deuxièmement, la coexistence de diverses tailles de femelles pourrait constituer une stratégie stable d'évolution. Selon la première explication, les femelles de tailles autres que la taille optimale devraient montrer une moins bonne adaptation tandis que, selon la deuxième, les femelles de toutes tailles devraient présenter des conditions également bonnes. Nous avons examiné les facteurs qui affectent la survie des oeufs, des alevins et des smolts de saumon coho (*Oncorhynchus kisutch*), dans le ruisseau Carnation, sur l'île de Vancouver, dans le but de déterminer l'état relatif des femelles de diverses tailles. Pour la mortalité de l'oeuf à l'alevin, c'est un modèle qui comportait seulement les effets de l'affouillement du lit de la rivière et de la qualité du gravier qui donnait la meilleure explication. Le fait d'y inclure l'effet de la taille des femelles, exprimé par la profondeur de l'enfouissement des oeufs, a nui à la capacité de prédiction du modèle. Nous n'avons trouvé aucune donnée montrant que les oeufs des femelles de plus grande taille présentaient de façon constante un taux de survie meilleur pendant l'incubation que ceux des femelles de plus petite taille. En fait, dans trois cas, il semble que les oeufs des petites femelles avaient un meilleur taux de survie. Dans le ruisseau Carnation, les gros smolts de 1 et 2 ans ne présentaient pas de façon constante un taux de survie en mer meilleur que celui des petits smolts. Nous n'avons donc pas pu démontrer que le succès de reproduction des femelles de grande taille était régulièrement plus élevé que celui des petites femelles, contrairement à l'hypothèse selon laquelle la taille des femelles serait le résultat d'un processus d'optimisation. Dans le ruisseau Carnation, la gamme de taille des femelles représente probablement une stratégie stable d'évolution dans laquelle toutes les tailles présentent des conditions égales. Nous proposons un modèle qui prédit la taille des femelles et sa variance en fonction des effets sélectifs concurrents de la qualité du gravier, de l'affouillement et de la concurrence pour les nids.

Received October 10, 1985
Accepted June 27, 1986
(J8483)

Reçu le 10 octobre 1985
Accepté le 27 juin 1986

In 1981, Roff observed that size was one of the most striking characteristics of a species, yet it was also a characteristic that had received relatively little investigation. Until recently this statement was true of Pacific salmon (*Oncorhynchus* spp.), but in the last few years a number of papers have appeared exploring various questions relating to the size of adult salmon. Ricker and his co-workers (Ricker 1980a, 1980b, 1981, 1982; Ricker et al. 1978; Ricker and Wickett 1980) analysed long-term changes in the size of maturing salmon and speculated about the possible genetic effects of

size-selective fisheries. Healey and Heard (1984) and Healey (1986) compared relationships among size, age, and fecundity in chinook (*O. tshawytscha*) and sockeye (*O. nerka*) salmon with predictions of life history theory. Gross (1984, 1985), van den Berghe and Gross (1984), and van den Berghe (1984) investigated relationships between size and reproductive success in male coho (*O. kisutch*) and between parental size and the survival probability of offspring in female coho.

A conclusion drawn from these studies is that, in undisturbed populations, large size confers a significant reproductive advantage to females and, under certain circumstances, to males as well. Healey and Heard (1984) and Healey (1986), for example, noted that chinook and sockeye salmon females appear to have sacrificed fecundity for size in the allocation of surplus energy. van den Berghe and Gross (1984) observed that larger female coho salmon buried their eggs deeper in the gravel, thus protecting them from superimposition and scour. Gross (1984, 1985) demonstrated the superiority of large male coho in contests for mates, although very small "jack" males could achieve equal fitness by "sneaking" provided they were not too abundant. van den Berghe (1984) demonstrated that the offspring of large females enjoyed a survival advantage at all life stages.

Apparently contradicting the demonstrable advantages of large adult size in female Pacific salmon, however, is the continued presence of a wide range of females sizes within spawning populations (i.e. range > mean/2). Size and growth rate have a relatively high heritability in salmonids (Ricker et al. 1978). If selection consistently favoured large size, we would expect most females to be large and the range of adult sizes in any population to be small. It appears that there may be conditions that favour small adult female size, just as there are conditions that favour small adult male size. Although this possibility has been noted (van den Berghe and Gross 1984; Healey and Heard 1985), it has not been examined very thoroughly.

The life history literature suggests two likely explanations for a wide range of female sizes. One assumes that an optimum size/age combination exists toward which natural selection is pushing the population. Variation in size of individuals under this hypothesis is determined by individual success in obtaining food (Stearns and Crandall 1984). High additive genetic variance could be maintained in the population under this hypothesis if the optimum size/age combination was driven by environmental conditions which were unstable (MacKay 1980). The second explanation ascribes variation in size to the existence of a stable, mixed reproductive strategy (evolutionary stable strategy or ESS (Maynard Smith 1982), but cf. also van Valen (1965)) in which different sized females adopt different reproductive strategies in a spatially variable reproductive environment. Under the first hypothesis, females that were not of optimum size would have lower fitness than those that were. In particular, considering the demonstrated advantages to large size in Pacific salmon, we would expect small females to have low fitness. Frequency-dependent selection should not occur under this hypothesis (Stearns and Crandall 1984). Under the second hypothesis, the fitness of large and small females should be equal, but selection for large and small females should be frequency dependent (Maynard Smith 1982).

In this paper we analyse data for the coho population in Carnation Creek on Vancouver Island with a view to determining whether large females are more fit than small females. We shall assess this by determining whether the progeny of large females enjoy a survival advantage at any stage between fertilization and adulthood.

Vital statistics of the Carnation Creek coho population have been collected since 1970 as part of a long-term study of the effects of logging on stream-dwelling salmonids (Hartman 1982). Female size has varied considerably both within and between years in this population, while egg-to-fry survival, fry and smolt size, and smolt-to-adult survival have varied considerably between years. This 12-yr data set provided us with a unique opportunity to investigate the effects of female size on the survival probability of their offspring and thus to test whether or not the range of female coho sizes might represent a mixed ESS.

Study Site and Methods

Carnation Creek (48°55′N; 125°00′W) flows into the south side of Barkley Sound on the west coast of Vancouver Island, B.C., approximately 70 km from Port Alberni. The stream is about 7.8 km long and drains a watershed of 10 km^2. Coho spawn throughout the accessible stream length, which is approximately 3070 m. The watershed is typified by coarse shallow soils, high annual precipitation (250–350 cm), most of which falls in the winter, little snowfall (<5% of total precipitation), and widely varying discharge (0.02–59.4 m^3/s). The Carnation Creek watershed has been partially clearcut logged.Logging began in the winter of 1975–76 and ceased in the winter of 1980–81, by which time 41% of the watershed had been clearcut (Dryburgh 1982).

A number of physical and biological measurements that are relevant to our investigation have been collected routinely throughout the Carnation Creek study. Stream stage height was continuously measured by a Stevenson recorder at a broad-crested hydrological weir near the stream mouth. This weir was operated by Inland Waters Directorate, Environment Canada. Stage heights were converted to discharges through a periodically calibrated ratings curve. Stream temperature was continuously recorded at the same site by a Lambrecht recorder. Forty-five samples of spawning gravel were taken each year at five sites distributed from 200 to 2250 m above the estuary (Scrivener and Brownlee 1982). The gravel samples were taken in areas of active spawning by the acetone – dry ice frozen core technique (Ryan 1970). Sampling locations were the same throughout the study. Each sample was partitioned into surface to 14-cm and 14- to 30-cm depth strata and particle size composition for each stratum obtained by dry sieving through meshes of 9.55, 2.38, 1.19, 0.297, and 0.074 mm. These sieves emphasize the, presumably, more biologically important finer fractions (Scrivener and Brownlee 1982). The Fredle index of gravel quality was derived from the size fraction data following the methods of Lotspeich and Everest (1981). We used only the size composition of the lower stratum in this study, since we found in preliminary analyses that the composition of the top stratum, which had the higher Fredle index, was uncorrelated with coho egg survival.

Returning adult coho salmon have been enumerated at a fence near the stream mouth from mid-September to March each year since 1971 (Andersen 1983, 1984, unpubl. data). Fork lengths to the nearest centimetre, sex, and scale age (including freshwater age) were recorded for each fish arriving at the fence. The majority of females entered the stream between the last week of October and the first week of November (Holtby et al. 1984).

TABLE 1. Mean fork length and range in length of females and downstream migrant fry, and numbers and mean fork length of yearling and 2-yr-old smolts in Carnation Creek arranged by brood year. A dash indicates data not available.

	Females		Fry		1+ smolt		2+ smolt	
Year	Length (cm)	Range	Length (mm)	Range	*n*	Length (mm)	*n*	Length (mm)
1971	—	—	38.0	33–45	1115	77.6	1444	99.2
1972	68.6	48–78	37.9	33–42	1214	74.1	874	94.6
1973	66.8	47–80	37.9	30–44	1247	75.2	1068	92.8
1974	66.9	49–76	38.7	34–42	1995	73.0	935	97.5
1975	65.9	42–79	38.1	35–43	1625	78.1	778	111.0
1976	63.5	50–74	37.9	34–40	3868	83.6	668	96.3
1977	65.2	53–76	36.4	33–42	2862	80.6	991	104.6
1978	65.3	49–75	37.0	33–43	3576	85.4	396	104.1
1979	64.0	44–77	37.5	33–45	3719	82.4	622	100.1
1980	66.8	51–75	37.4	32–41	2847	86.6	305	103.0
1981	64.4	43–79	37.2	34–41	3437	86.1	119	102.2
1982	64.4	50–76	36.5	31–40	2984	84.0	—	—
1983	61.4	46–74	34.6	30–40	—	—	—	—

The total number of fry and smolts migrating downstream in the spring was enumerated at the fence from mid-March through August each year. In mid- to late May each year the total number of coho fry resident in the creek was censused in six sections, each 32–113 m long, by intensive pole seining and electroshocking (Andersen 1983, 1984, unpubl. data). Catches for all sections were pooled and the number in all sections was estimated by the removal method (Seber and LeCren 1967). Total stream population was obtained by multiplying the estimate for sections censused by the ratio of uncensused to censused stream length.

For the purpose of this study, we assumed that the total number of emergent fry each year was the number of fry resident in the creek at the time of this census plus the number that had migrated past the fence prior to the census. Without doubt, emerging fry experienced mortality so that we have consistently underestimated the true number of emerging fry. We have assumed, however, that the magnitude of this unknown bias was randomly distributed among years (e.g. see Hartman et al. 1982).

During the spring outmigration of fry and smolts, samples of several hundred migrating fry were taken periodically, usually every 7–10 d, and their fork lengths measured to the nearest millimetre. The frequency distribution of fry lengths that we used in this study were the averages of samples taken on the three dates closest to the median migration date, weighted by the total number of fry captured on each sampling date. Since resident and migrant fry were not significantly different in size in Carnation Creek (Hartman et al. 1982), we presume that these measurements are representative of the population. All smolts captured were measured to the nearest millimetre and, periodically, samples of scales were taken to determine a size–age relationship that was applied to all smolts of that year.

The spawning population in Carnation Creek was too small to permit females to be sacrificed for fecundity estimation. Consequently, we used fecundity data for the 1975 brood from the nearby Robertson Creek hatchery (second-generation hatchery coho). Thirty-eight females spanning as wide a range of size as possible had been selected from the hatchery holding pens and their fork lengths, total ovary weights, and fecundities measured. We augmented these data by measurements from four Carnation Creek females that had died at the counting fence.

Female weights were not measured at Carnation Creek. We therefore estimated their weight by a regression derived from measurements of length and weight for female coho returning to Rosewall Creek on the east side of Vancouver Island in 1975 (T. Bilton, Department of Fisheries and Oceans, unpubl. data):

$$\log \text{weight (g)} = 3.3183\,[\log \text{fork length (mm)}] - 5.843.$$

(Note that $\log_{10}$ is the transformation used throughout this paper.)

To determine within-cohort effects of smolt size on marine survival, we compared, for the adult return years 1973, 1979, and 1983, the size frequency distribution of outmigrating smolts 1 yr earlier with the calculated sizes at smolting of the returning adult females. We chose adult return years 1973 and 1983 arbitrarily as early and late return years in the study, and we chose adult return year 1979 because the large number of female returns in that year permitted us to examine relative marine survival of both yearling and 2-yr-old smolts. Returns of 2-yr-old smolts were too few in 1973 and 1983 to permit us to determine their within-cohort survival.

Outmigrating smolts for these cohorts were measured at the counting fence as described earlier, and scales were taken from a randomly selected subsample. Scales were removed from the left side immediately posterior to the dorsal fin and above the lateral line and smeared onto a labeled glass slide. We measured, under 45× magnification, the radii of 2–5 scales/smolt along a line 20° ventral to the anterior–posterior axis of the scale. The ages of the smolts were also recorded. From measurements of smolts and smolt scales for smolt years 1972, 1976–79, and 1982–83, we determined the following predictive relationship for smolt fork length:

$$\text{FL(mm)} = 31.1 + 108.4 \times \text{scale radius (mm)} + 10.1 \times \text{age} \quad (r^2 = 0.774;\ \text{SE}_{\text{est}} = 6.557;\ n = 640).$$

Scales were sampled from the same body location on returning adults in the three years noted above. We made measurements on adult scales from plastic impressions at 45×

magnification following the same procedure as for the smolt scales. We measured the distance from the focus to (1) the edge of the freshwater growth zone (the nuclear scale, Clutter and Whitesel 1956), (2) the ocean annulus, and (3) the scale edge for two (sometimes only one) scales from each female and averaged the measurements. We then calculated the smolt size for each returning female by the relationship given above.

We tabulated the fork lengths of outmigrating smolts in 5-mm size groups (45–120 mm for yearling smolts, 70–150 mm for 2-yr-old smolts) and scaled the frequencies to equal the total number of returning adult females in each of the years. These expected frequency distributions were compared with the observed frequency distributions calculated from the female scales by a chi-square test for goodness of fit.

It is possible that the observed variability in female size is due to environmentally induced variation in individual growth rates. To examine this possibility, we looked for a relationship between scale width from the edge of the freshwater growth zone to the ocean annulus and from the ocean annulus to the scale edge. The scale widths serve as indices of growth over the first and second year in the ocean, respectively. If the observed variation in female size was due mostly to spatial heterogeneity in the ocean, which determined the feeding success of individual females, then we would expect no relationship between these indices of growth for individuals during their two ocean years. Alternately, if the variability in female size was due to the expression of differences in the growth potentials of individual fish, then we would expect a positive correlation between scale widths in sequential years.

We also compared the relative ocean survival of small (yearling) and large (2-yr-old) smolts over all years of the study. Although this comparison involves fish from different cohorts, it provides us with a way to examine relative survival over a wider range of smolt sizes and years.

Results

Vital Statistics of the Population

The number of spawning females in Carnation Creek has ranged from a low of 39 in 1981 to a high of 176 in 1979 and has averaged 72. Their average length has ranged from a low of 61.4 cm in 1983 to a high of 68.6 cm in 1972 (Tables 1, 2). Overall length has ranged from 42 to 80 cm and the length frequency distribution shows a slight positive skew (Table 1; Fig. 1). When average female size was small (e.g. 1983, Fig. 1), the size frequency distribution tended to be symmetric, whereas positive skew was most pronounced when average female size was large (e.g. 1972, Fig. 1). The range of female sizes present in the population has varied little from year to year.

Average fry length has ranged from 34.6 mm in 1983 to 38.7 mm in 1974, and the number of fry produced each year has ranged between 7000 and 59 000 (Table 2). Yearling and 2-yr-old smolts were not very different in abundance during the early years of the study, but since 1974 yearling smolts have greatly outnumbered 2-yr-olds (Table 1). Smolt production by brood year has ranged from 2088 to 4536 over the years for which we have data. Yearling smolts have ranged 73–86.6 mm in average fork length and 2-yr-old smolts have ranged 92.8–111.0 mm in average fork length (Table 1).

The fecundity on length regression calculated from the combined Robertson and Carnation creeks females was

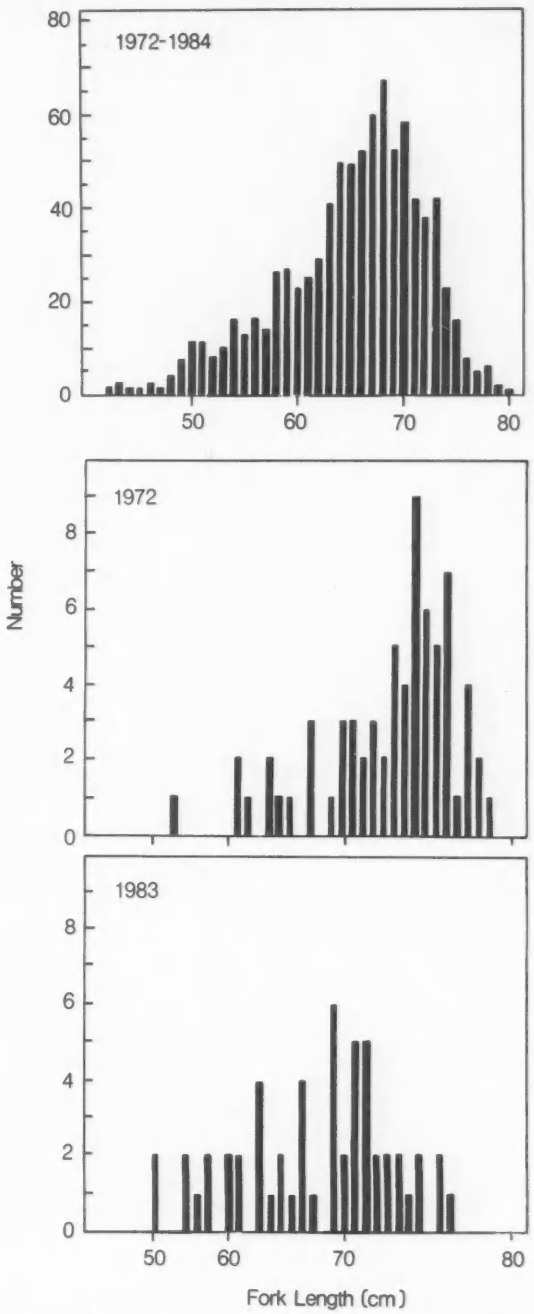


FIG. 1. Size frequency histogram of all female coho measured at the counting fence on Carnation Creek between 1972 and 1984, and histograms for the individual years with the largest (1972) and smallest (1983) females.

$$\log \text{fecundity} = 2.9140\,[\log \text{fork length (cm)}] - 1.9514.$$

The four Carnation Creek females had fecundities well within the range for Robertson Creek females of similar length, sug-

TABLE 2. Number of females, potential egg deposition, fry production, egg-to-fry mortality, peak discharge during incubation, and Fredle index of gravel quality in Carnation Creek by brood year.

Year	Females	Potential egg deposition	Fry production	Egg-to-fry mortality	Winter discharge (cm)	Fredle index
1972	69	177 248	35 121	0.8019	30.3	3.64
1973	71	169 038	37 444	0.7785	18.8	3.54
1974	65	154 661	44 808	0.7103	19.1	3.54
1975	69	160 674	59 019	0.6327	17.8	3.66
1976	54	111 169	23 465	0.7889	34.9	3.15
1977	51	112 251	39 717	0.6462	9.8	4.30
1978	56	124 365	30 529	0.7545	22.5	3.45
1979	176	369 610	47 542	0.8714	23.2	3.54
1980	64	151 283	17 093	0.8870	39.2	3.34
1981	39	84 572	12 473	0.8525	50.0	3.28
1982	70	150 007	25 900	0.8273	36.2	3.18
1983	51	101 569	7 181	0.9293	59.5	2.99

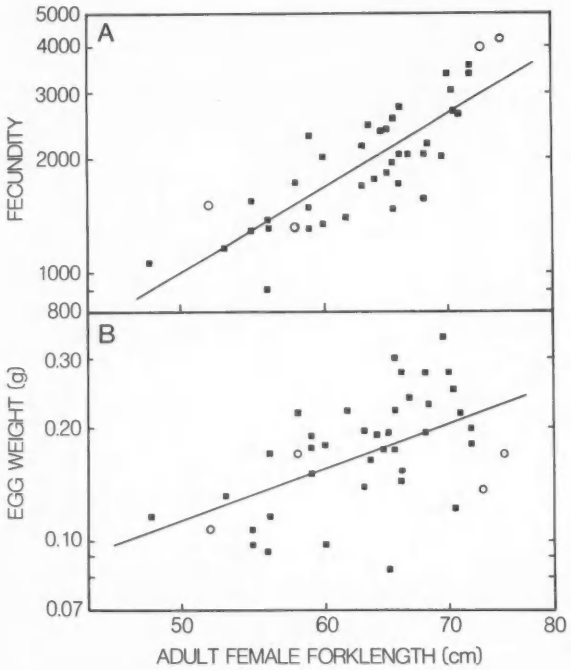


FIG. 2. Relationship between (A) fecundity and fork length and between (B) egg weight and fork length for Robertson Creek (■) and Carnation Creek (○) females.

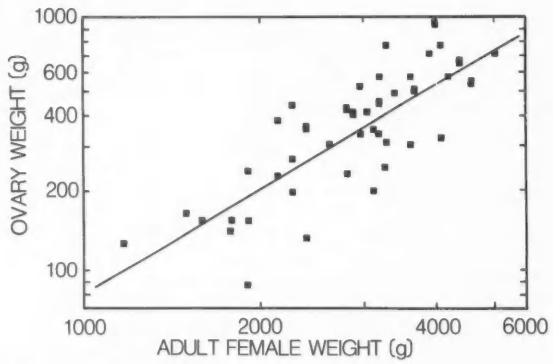


FIG. 3. Relationship between ovary weight and fork length for Robertson Creek females.

gesting that the Robertson Creek fecundity measurements were representative of Carnation Creek (Fig. 2A).

The fecundity on length regression above is similar to another published relationship for coho (Allen 1958) although quite different from a fecundity on length regression for Robertson Creek published by Beacham (1982), who used a linear function. There is no doubt, however, that the true relationship is a power function.

Egg weight also increased significantly with female size, and the relationship was (Fig. 2B)

$$\log \text{egg weight (g)} = 1.7479\,[\log \text{fish length (cm)}] - 3.9101.$$

Another relationship that will be important to the interpretation of size and fecundity in Carnation Creek coho is that between female size and mature ovary weight. For Robertson Creek coho this regression was (Fig. 3)

$$\log \text{ovary weight (g)} = 1.4049\,[\log \text{fish weight (g)}] - 2.3270.$$

Female weights used for this regression were estimated from their lengths by the length on weight regression for Rosewall Creek coho given earlier. The slope greater than 1.0 indicates that mature ovary weight is a greater proportion of total body weight among large than among small females.

Effects of Female Size on Egg-to-Fry Survival

From the fecundity on size regression and the sizes of females spawning in Carnation Creek we calculated the potential egg deposition for each brood year (Table 2). We estimated egg-to-fry mortality as 1 minus the ratio of emergent fry to potential egg deposition. As we noted in the methods, these

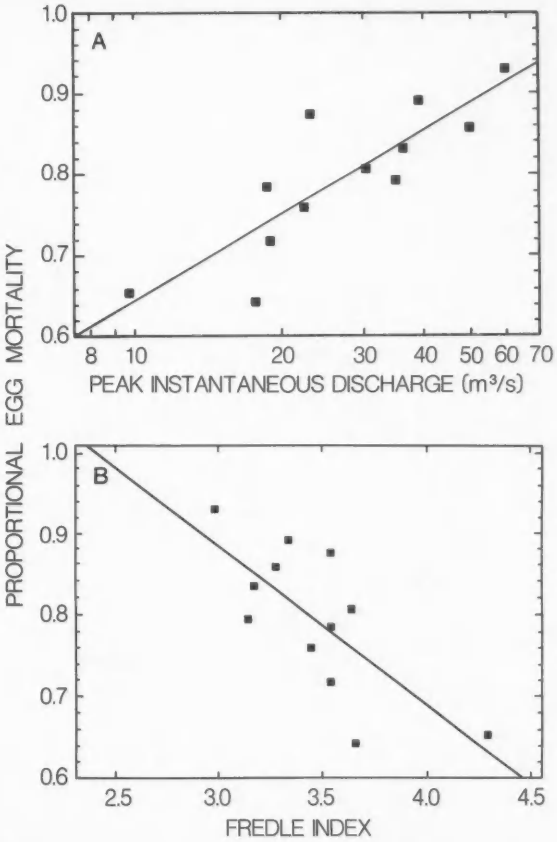


FIG. 4. Relationship between (A) egg-to-fry mortality and instantaneous peak winter discharge and between (B) egg-to-fry mortality and Fredle index in Carnation Creek.

mortality estimates are biased high. Egg-to-fry mortality for the broods 1972–83 has ranged from a low of 64.1% (1975 brood) to a high of 93.1% (1983 brood) (Table 2). Mortalities in this range are typical of other salmonids in Pacific coast streams (McNeil 1966; Phillips 1971).

van den Berghe and Gross (1984) suggested that redd superimposition was an important cause of egg mortality in coho. The maximum number of females in any brood year at Carnation Creek was 176, and the spawning fish were distributed throughout 3 km of accessible stream. We believe, therefore, that redd superimposition was unlikely to have been a serious cause of egg-to-fry mortality in Carnation Creek. The absence of frequent physical disturbance of redds by other spawners in the stream is also indicated by the fact that there was no significant relationship between egg-to-fry mortality and either the number of female spawners ($r = 0.216$, $p > 0.70$) or total egg deposition ($r = 0.155$, $p > 0.70$). This does not mean, however, that females did not compete for nest sites.

Egg-to-fry mortality was significantly positively correlated with the logarithm of the peak instantaneous discharge during the period of egg incubation (November 16 to February 15) ($r = 0.843$, $p < 0.01$, Fig. 4), suggesting that scour may have been an important cause of mortality. The logarithmic transformation was required to linearize the relationship. Egg-to-fry mortality was also significantly negatively correlated with the Fredle index of gravel quality ($r = -0.730$, $p < 0.01$, Fig. 4). Decreasing values of the Fredle index indicate decreasing mean particle size (Lotspeich and Everest 1981) and, by inference, deteriorating gravel quality. Thus, egg mortality appears to depend on gravel quality as well as on scour. There was no simple correlation between egg mortality and mean female size ($r = -0.410$, $p > 0.05$).

To explore the relationship between egg-to-fry mortality and peak discharge, gravel quality, and female size, we developed a number of simple causal models that assumed an explicit relationship between mortality and discharge and gravel quality. Our objective was to quantify the effect of adult female size on egg-to-fry mortality relative to the other factors. Consequently, some of the causal models assumed that female size had an effect on the depth of egg deposition whereas others did not. We determined the success of each model by comparing the interannual pattern of egg-to-fry mortality predicted by the model with the observed pattern. With regard to the hypotheses we are testing, the optimization hypothesis would be favoured if models containing an effect of female size gave better predictions of the interannual pattern of egg-to-fry mortality, whereas the mixed ESS hypothesis would be favoured if models that ignored female size gave just as good prediction as those that did not.

We used this approach rather than a multiple regression analysis of our data for a number of reasons. We were not trying to predict egg-to-fry mortality per se, an objective that would be better addressed through regression modelling. Rather, we were trying to determine if female size was a necessary variable in any model explaining variation in egg-to-fry mortality. Since our data were observational and not experimental, we could not assert that a regression model calculated from the data had any particularly causal interpretation (Gilbert 1973). Furthermore, even if we knew beforehand that gravel quality, peak discharge, and female size were the principal determinants of egg-to-fry mortality, a multiple regression model incorporating those variables would still be difficult to interpret. For example, the independent variables in the data set are all intercorrelated and consequently their regression coefficients might not reflect their relative importance. Also, the effects of peak discharge and gravel quality on egg-to-fry mortality are not additive, thereby violating a basic assumption of the regression model. The causal models that we developed avoid these problems, although they do demand that we make assumptions about the functional relationship between egg-to-fry mortality and peak discharge, gravel quality, and female size.

In developing the models, we first devised a relationship between scour depth and peak discharge. We assumed that the correlation between egg mortality and peak discharge stemmed from an underlying relationship between scour and egg loss, and that the scour depths that occur in Carnation Creek at peak discharge are sufficient to account for significant egg mortality. Unfortunately, scour has not been regularly measured at Carnation Creek. Reliable measurements were available for 1983–84, the winter of greatest peak discharge. Scour during this winter averaged 14 cm ($n = 18$, range 0–29 cm; Les Powell, Department of Fisheries and Oceans, Vancouver, B.C., unpubl. data). Qualitative observations over the 12 yr of the study indicated that bed load movement in Carnation Creek begins at a discharge of about 4 m^3/s. In three streams in Oregon, scour depth was linearly related to the logarithms of

TABLE 3. Comparisons of predictions by seven models of egg-to-fry mortality with observed egg-to-fry mortality in Carnation Creek. Criteria for perfect prediction are a slope of the regression of observed on predicted mortality of 1 and an intercept of 0. Egg depth distributions: type 1, uniform 5–25 cm; type 2, uniform 5 cm, depth dependent on female size; type 3, stratified 5-cm band at depth dependent on female size. See text for further details of model formulation.

Model No.	Scour	Gravel quality	Egg depth deposition	Regression slope (b)	Prob. $b = 1$	Regression intercept (a)	Prob. $a = 0$	Correlation coefficient
1	−	+	None	1.795	>0.100	−0.071	>0.500	0.729
2	+	−	Type 1	0.610	<0.050	0.640	<0.001	0.844
3	+	+	Type 1	0.801	>0.200	0.306	<0.050	0.825
4	+	−	Type 2	0.340	<0.001	0.643	<0.001	0.829
5	+	+	Type 2	0.541	<0.010	0.412	<0.001	0.821
6	+	−	Type 3	0.368	<0.001	0.745	<0.001	0.664
7	+	+	Type 3	0.611	<0.050	0.462	<0.010	0.759

average daily discharge (Moring 1975). Using this form for the relationship and, as two end points, the discharges for no scour (4 m^3/s) and 14-cm scour (59.5 m^3/s), we calculated the scour depth − discharge relationship as

$$\text{Scour depth (cm)} = 11.9\ [\log \text{peak discharge } (m^3/s)] - 7.19.$$

We emphasize that this is not an empirically determined relationship but a hypothetical one which constitutes one of the assumptions in our models.

Egg-to-fry mortality presumably varies as a function of gravel composition independently of physical disturbance. As mean particle size decreases, percolation rates and O_2 concentrations fall, resulting in increased rates of physiological mortality. Furthermore, as pore size decreases, alevin movements become more restricted and emergence success is reduced (Phillips et al. 1975). We used the relationship

$$\text{Egg mortality} = -0.112\ (\text{Fredle index}) + 0.868$$

from Platts et al. (1983) to estimate egg mortality resulting from gravel composition alone.

We assumed three possible patterns of egg distribution within the gravel. In distribution 1, the eggs were uniformly distributed from 5 cm below the surface down to 25 cm. The 5-cm upper depth was arbitrary but the 25-cm lower depth was the deepest that van den Berghe and Gross (1984) observed coho to bury their eggs in Deer Creek Junior. In distribution 2, the eggs were distributed uniformly from 5 cm down to a maximum depth determined by the size of females in the spawning population. These maximum depths were taken from the female size − nest depth relationship in van den Berghe and Gross (1984). In distribution 3, each female deposited her eggs in a 5-cm-wide band, the lower limit of which was determined by the van den Berghe and Gross (1984) female size − nest depth relationship.

We constructed seven models using these relationships. In model 1, gravel quality alone determined egg-to-fry mortality. In model 2, egg distribution 1 was employed and all mortality was due to scour. In model 3, egg distribution 1 was employed and egg-to-fry mortality was due to both scour and gravel quality. In models 4 and 5, egg distribution 2 was employed. Scour was the only mortality effect included in model 4 whereas both scour and gravel quality effects were included in model 5. In models 6 and 7, egg distribution 3 was employed. Scour was the only mortality effect included in model 6 whereas both scour and gravel quality effects were included in model 7. In calculating mortality due to scour in the models, we assumed that all eggs shallower than the depth of scour were killed.

The criterion we used to judge the success of each model as a predictor of egg mortality was the slope of the regression of observed on predicted mortality. A slope of 1 indicated that the model was adequately capturing interannual variation in egg-to-fry mortality. A slope different from 1 indicated that the model was making inappropriate predictions at either or both the high or low end of the mortality distribution. A positive intercept for the regression, associated with a slope of 1, indicated that the model had captured interannual variation but that some component of egg-to-fry mortality was unexplained by the model.

Of the seven models, both model 1, which assumed only the mortality effects of gravel quality, and model 3, which assumed a uniform distribution of eggs with depth and the combined mortality effects of scour and gravel quality, yielded regression slopes not significantly different from 1 (Table 3). Of these, model 3 accounted for more variance than model 1 and predicted mortality rates closer to those actually observed. All other models yielded slopes that were significantly less than 1 (Table 3).

Although it gave the best fit, model 3 nevertheless had a positive intercept. This indicates that there was a constant (on average) mortality source that was not accounted for by the model.

Of particular significance is the fact that the four models that explicitly assumed that female size was important in determining egg-to-fry mortality (models 4–7) gave inadequate predictions of mortality. Apparently any effect of female size on the depth to which eggs were buried in the gravel was unimportant to interannual variations in egg mortality in Carnation Creek.

The performance of model 3 can be degraded if we assume that the eggs are uniformly distributed to a lesser maximum depth. Such an assumption is equivalent to placing more weight on scour as a mortality cause. Alternatively, if the depth of egg burial is increased, then increased weight is given to gravel quality in the model. When gravel quality is the only mortality factor considered (model 1), the slope of observed mortality regressed on predicted mortality is greater than 1, although not significantly so (Table 3).

We looked for correlations between the egg mortality unexplained by model 3 and 10 physical and biological variables (average stream temperature from November 16 to February 15, duration of flow less than 0.25 m^3/s and duration of flow

TABLE 4. Comparison of observed frequency distributions of fry length (mm) with those predicted by the model relating female size to egg and fry size described in the text. Observed mean fry sizes that are significantly different from the predicted are shown by eigher "<" or ">" indicating the direction of the difference. Slopes that are much less than 1 (e.g. 1977, 1983) indicate that the slopes of the observed and predicted size frequency curves were very different.

	Mean fry length			Regression: observed on predicted		
Year	Predicted (95% CI)		Observed	Slope	Intercept	r
1972	38.4 (37.5–39.0)		37.9	0.700	0.0188	0.884
1973	38.0 (37.2–38.7)		37.9	0.690	0.0174	0.835
1974	37.9 (37.1–38.7)	<	38.7	0.821	0.0111	0.857
1975	38.1 (37.2–38.7)		38.1	1.123	−0.0077	0.891
1976	37.2 (36.5–38.1)		37.9	0.986	0.0009	0.946
1977	37.5 (36.7–38.3)	>	36.4	0.433	0.0354	0.483
1978	37.5 (36.8–38.4)		37.0	0.834	0.0104	0.968
1979	37.3 (36.9–37.8)		37.5	0.780	0.0134	0.883
1980	37.9 (37.1–38.6)		37.4	0.765	0.0147	0.817
1981	37.7 (36.5–38.5)		37.2	0.730	0.0171	0.734
1982	37.4 (36.8–38.2)	>	36.8	0.751	0.0051	0.751
1983	36.8 (36.1–37.7)	>	34.6	0.218	0.0489	0.276

less than 4 m^3/s during the same period, estimated scour depth, peak discharge, gravel quality, total numbers of female spawners, mean female fork length, estimated mean egg weight, and year). None of these variables was significantly correlated with the unexplained mortality. Number of females had the highest correlation with unexplained mortality (r = 0.563, p = 0.057) and this correlation was marginally significant. The strength of this correlation was due entirely, however, to the point for 1979 when females were almost three times more abundant than the long-term average (Table 2). Unexplained mortality was also exceptionally high in this year, perhaps indicating that some redd superimposition occurred. Alternatively, competition for nest sites may have forced many females to spawn in poor nesting locations.

Relative Survival of Eggs of Different Sizes

Another way of assessing the relative success of offspring of different sized females is to examine the size of fry produced. Fowler (1972) has shown that larger chinook salmon eggs produce larger fry and fingerlings, and we observed that larger coho females produced larger eggs (Fig. 2B). In spite of the indication from our models that female size made no contribution to interannual variation in egg-to-fry mortality, perhaps the eggs of larger females survived better in years of high discharge, and this would be revealed in the size of fry produced.

We were unable to locate any published relationships between coho female size and the size of their emergent fry. We assumed, however, that such a relationship exists and estimated it in three slightly different ways. First, using all 12 years of data from Carnation Creek, we regressed the smallest, largest, and mean observed fry fork lengths from each year on the corresponding estimates of egg weight for the smallest, largest, and mean female fork lengths. Second, also using all 12 years of data, we regressed the 10%, 90%, and mean fry fork lengths for each year on the corresponding estimates of egg weights for the 10%, 90%, and mean female fork lengths. Third, using an early year (1974) selected for average female size, low flow, and high gravel quality, we regressed the 10%, 20%, . . . , 90% fry fork lengths on the corresponding egg weights for the 10%, 20%, . . . , 90% female fork lengths. The resulting regressions were

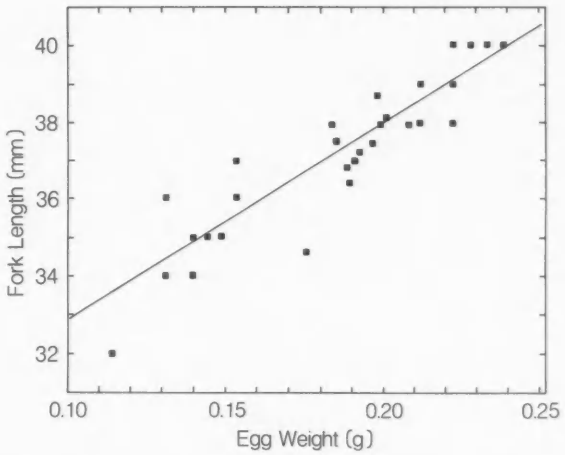


FIG. 5. Relationship between fry length and egg weight derived for Carnation Creek.

(1) Fry fork length (mm) = 46.79[egg weight (g)] + 28.76

(2) Fry fork length (mm) = 51.31[egg weight (g)] + 27.74

(3) Fry fork length (mm) = 49.49[egg weight (g)] + 28.55.

All three regressions were significant and indistinguishable. We chose the second, arbitrarily, as the predictive relationship (Fig. 5). From this relationship we are able to calculate the expected fry size distribution for each year by summing, across all females within the year, the product of individual fecundities and estimated fry lengths. We employed the error terms in the fecundity on female size, egg weight on female

TABLE 5. Numbers of yearling and 2+ smolts migrating to sea each year and female adults produced by those smolts (i.e. returning to spawn one year later) in Carnation Creek. The ratios of yearling: 2+ smolts in seaward migrants and returning females provide a measure of the relative ocean survival of the two smolt age classes (see text for details).

Year of seaward migration	Smolts		Returning females		Ratio 1+:2+ among	
	1+	2+	1+	2+	smolts	females
1971	1062	1415	39	36	0.751	1.083
1972	1346	574	62	13	2.345	4.769
1973	1115	776	26	40	1.437	0.650
1974	1214	1444	27	42	0.841	0.643
1975	1247	874	22	33	1.427	0.667
1976	1995	1068	25	26	1.868	0.962
1977	1625	935	34	22	1.738	1.545
1978	3868	778	148	28	4.972	5.286
1979	2862	668	63	8	4.284	7.875
1980	3576	991	36	7	3.608	5.143
1981	3719	396	61	10	9.391	6.100
1982	2847	622	46	5	4.577	9.200

size, and fry size on egg weight relationships to generate 500 Monte Carlo simulations of predicted fry size for each year. The range of predicted fry sizes so generated provided upper and lower confidence limits of predicted mean fry size against which to compare the observed mean fry size. We also compared the predicted distributions of fry length with the observed lengths of fry migrating downstream in the spring. As we noted earlier, comparison of samples of resident fry captured in the creek with those captured at the fence showed that there was no difference in the size of resident and migrating fry during the period of the main fry run (Hartman et al. 1982). If the progeny of large females experienced a survival advantage, then we would expect to observe fry that were larger than the predicted range of mean fry size in some years, particularly years of high discharge.

As we noted earlier, the observed mean fork lengths of outmigrating fry in Carnation Creek varied considerably among years from a low of 34.6 mm in 1983 to a high of 38.7 mm in 1974 (Table 1). The mean fork lengths of fry were correlated with both adult female fork length ($r = 0.720$, $p < 0.01$) and with time ($r = 0.729$, $p < 0.01$). For most years the fry size distribution predicted from equation (2) closely matched the observed fry size (Table 4; Fig. 6A, 6B). In all but one year the predicted distributions were somewhat more peaked than those observed, which resulted in the slopes of the regressions within each year of observed on predicted fry lengths being slightly less than 1 (Table 4). For each of the 12 years, we calculated the distribution of the mean predicted fry lengths by Monte Carlo stimulation. In this simulation the effects of random error in the three regression relationships fecundity of female fork length, egg weight on female fork length, and fry fork length on egg weight were allowed for, and the 95% confidence intervals around the predicted mean fry fork lengths could be calculated. In three of the 12 years the observed fry fork lengths were significantly smaller than those predicted (1977, 1982, 1983; Table 5; Fig. 6C–6E), and in one year the fry were significantly larger (1974; Table 5; Fig. 6F). There was no evidence from the fry size data, therefore, that any survival advantage consistently accrued to the eggs of larger females.

In two of the three instances in which the progeny of small females survived significantly better than expected, the observed mean fry size was substantially smaller than the predicted mean size (1977, 1983; Table 4). In these two years the lowest (1977) and highest (1983) incubation stream discharges of the study were observed. When flow duration is considered, 1977 was, in fact, an even drier winter than is indicated by the low peak discharge. In the other two years in which we observed significant departures from predicted fry size, the differences between predicted and observed sizes ranged from 0.7 mm smaller (1982) to 0.8 mm larger (1974).

Relative Survival of Smolts of Different Sizes

One final assessment relating to the advantage of large female size may be made. If large females produce large fry which become large smolts, as suggested by van den Berghe (1984), they may ultimately experience higher ocean survival. Bilton et al. (1982) and Bilton (1984) documented greater ocean survival among larger smolts of both coho and chinook. Paloheimo and Elson (1974) observed that large female Atlantic salmon (*Salmo salar*) made a greater contribution than grilse females to smolt production. Although we have been unable to demonstrate that progeny of large females experience a survival advantage during incubation, it may still be the case that larger females produce larger fry and smolts and these survive better at sea. Carnation Creek smolts vary considerably in size, and the existence of two age classes of smolts each year accentuates the size variation (Table 1). Female smolts spend about 18 mo at sea and return to spawn in the fall of the year following their migration to sea. If larger smolts experienced a survival advantage, then we would expect that the survivors within each age class of smolts would be the large members of the cohort and that the ratio of yearling smolts to 2-yr-old smolts would be greater among seaward migrants than among adult females returning the next year.

As noted in the methods, we examined within-cohort marine survival in relations to smolt size for only three adult return years. The data were sufficient to examine survival of yearling smolts in all three years, but were sufficient for 2-yr-old smolts only in return year 1979. Marine survival varied significantly with smolt size for all cohorts examined. For return years 1979 and 1983, smolts smaller than average size survived better than

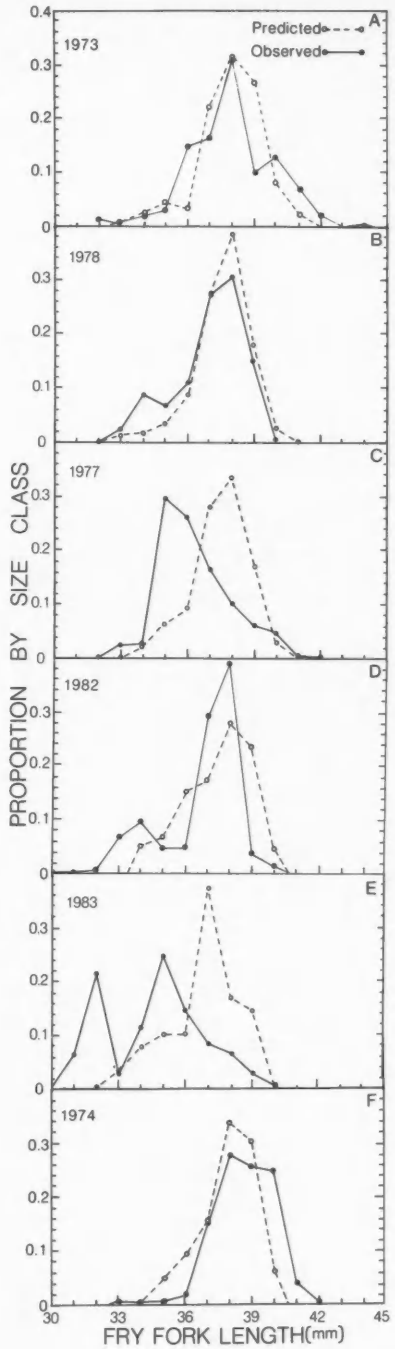


FIG. 6. Comparison of fry length frequencies predicted by a model relating egg size to fry size with observed fry length frequencies in Carnation Creek. (A and B) 1973 and 1978, examples of agreement between observed and predicted frequencies (typical for eight years); (C, D, and E) 1977, 1982, 1983, the years in which observed fry lengths were significantly smaller than predicted; (F) 1984, the year in which observed fry lengths were significantly larger than predicted.

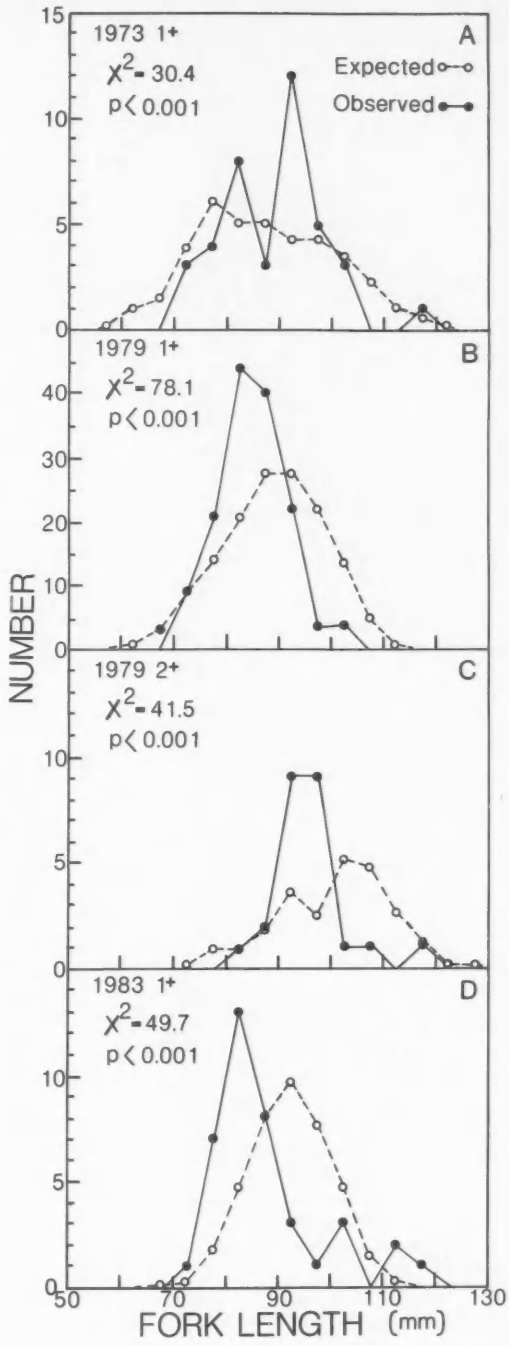


FIG. 7. Comparison of the smolt length frequencies observed in (A) 1972 (yearlings), (B) 1978 (yearlings), (C) 1978 (2-yr-olds), and (D) 1982 (yearlings) with smolt length frequencies back-calculated from scale measurements from females returning in (A) 1973, (B) 1979, (C) 1979, and (D) 1983. Shown are chi-square values and the probabilities that the observed and back-calculated frequency distributions were taken from the same population.

TABLE 6. Regressions of (A) adult female fork length (cm) on scale radius (mm) and (B) scale width (mm) for second ocean year on scale width for first ocean year by return year and smolt age.

			A				B			
Return year	Smolt age	n	Slope	Intercept	r	Prob. slope = 0	Slope	Intercept	r	Prob. slope = 0
1973	1	39	14.4	18.4	0.815	<0.001	0.426	0.588	0.338	<0.05
1979	1	143	9.27	35.4	0.658	<0.001	0.162	1.004	0.178	<0.05
	2	24	1.93	57.3	0.130	>0.2	−0.198	1.586	−0.259	>0.2
1983	1	40	7.33	40.8	0.459	<0.01	0.388	0.512	0.362	<0.05

those larger than average size. This was true of both age classes of smolts in return year 1979 (Fig. 7). For return year 1973, however, larger than average smolts survived better.

For all years, the size of adult females arising from yearling smolts was uncorrelated with their back-calculated size at smolting (all r values near 0.00). For the 1979 return year, the size of adult females arising from 2-yr-old smolts was negatively correlated with their back-calculated size as smolts, but the relationship was not statistically significant ($r = -0.379$, $p < 0.10$).

Thus, for the years selected, the tendency was for small smolts within a cohort to experience better marine survival. We can also make a tentative assessment of marine survival in relation to size across all years by comparing the survival probabilities of yearling and 2-yr-old smolts. We have 12 complete years of data with which to make the comparison between smolt ages (Table 5). For six of the 12 years the ratio of yearling to 2-yr-old smolts was greater among seaward migrants than among the returning adults, but for the other six years it was less (Table 5). There was no significant difference, overall, between the ratio of yearling to 2-yr-old smolts in seaward migrants and returning adult females (paired t-test, $t = 0.88$, $p > 0.05$). Thus, it appears that, for Carnation Creek, smolt size is not consistently an important factor in marine survival.

Environmental Variability and Female Size

Since adult female size was not related to smolt size, we examined the possibility that the variation in size of adult females within a cohort was due simply to variability in the ocean environment. We did this through a comparison of growth during the 2 yr each female spends in the ocean, as revealed by scale growth. The radius of an adult female's scale was correlated with her fork length, at least among yearling smolts (Table 6). We also observed that scale growth during the first ocean year ranged between 30 and 70% of total scale growth. With these relationships, and the observed distribution of female sizes in Carnation Creek, as constraints we would expect the following relationships between incremental scale widths (as an index of growth rate) in the first and second ocean years. (1) If the variation in adult female size was due to spatial heterogeneity in ocean conditions, and hence to chance variation in the feeding success of individual females, we would expect a *negative* correlation between scale widths in adjacent years. The only circumstance in which the expected negative relationship would not hold would be if the ocean feeding conditions were patchy and if individuals remained within the same patch throughout their ocean life. Since coho are known to range widely during their ocean life (Argue and Heizer 1974), this possibility is unlikely. (2) Alternatively, if the variation in adult female size is due to the expression of differences in growth potential of individuals, then we would expect a *positive* correlation between growth in adjacent years. Such a result would favour the hypothesis that size variation is being maintained in the population through inheritance of growth rates.

For yearling smolts in the three return years considered, growth in the second ocean year was significantly positively correlated with growth in the first ocean year (Table 6). The correlation for the 2-yr-old smolts was negative but not significant; however, the relationship between scale radius and female size was also not significant for 2-yr-old smolts (Table 6). The positive correlations for the yearling smolts were weak ($r^2 = 0.03-0.13$), indicating that there was a considerable environmental component to variation in female size. Nevertheless, there was a significant tendency for fish that grew slowly during their first ocean year also to grow slowly during their second ocean year and vice versa. This relationship is contrary to the relationship expected if all variation in female size was environmental.

Discussion

Carnation Creek females varied 38 cm in length, and although there tended to be more large females in the population, small females were by no means rare. This range in size is not unusual for coho (Healey 1986).

As has been observed for other species (Rounsefell 1957; Fowler 1972), large female coho produced larger eggs than small females. Large female coho were also, apparently, able to devote relatively more energy to egg production than small females, since their ovaries were a greater proportion of their body weight. These results suggest that large females may have experienced better feeding conditions and that at least some of the observed variation in female size was due to the feeding conditions encountered by individuals.

The slope of the fecundity on length regression was nearly 3, indicating that fecundity was directly proportional to weight in Robertson Creek (and presumably Carnation Creek) coho. This is in contrast with the slopes of these regressions for chinook and sockeye, which are much less than 3 (Healey and Heard 1984; Healey 1986), a result which suggested that chinook and sockeye had sacrificed fecundity for body size. Thus, there was no indication from either the ratio of ovary weight to body weight, or the slope of the fecundity on length regression, that coho had sacrificed egg production for body size. These observations are consistent with the hypothesis that variation in female size is a result of environmental variation and that

average size is determined by some optimizing process. Under this hypothesis, since female coho are aggressive and territorial during spawning, one would expect that large females would dominate on the spawning grounds and that small females would be less successful at producing offspring (cf. Hartman 1969). The balance of our observations do not satisfy this expectation.

The growth that returning females achieved in their first year in the ocean was positively correlated with that achieved in their second year in the ocean for yearling smolts in all of the three years that were examined. A significant component of the variation in adult female size is, therefore, likely due to the expression of differing growth potentials among the individual fish. The lack of any relationship between returning female size and back-calculated smolt size indicates, however, that size achieved before smolting is not an indicator of the growth potential of a fish in the ocean.

Interannual variation in egg-to-fry mortality in Carnation Creek was most successfully explained by a model that included only the effects of scour during the incubation period and gravel quality, factors that various other authors (McNeil 1966; Phillips 1971; Phillips et al. 1975; Lotspeich and Everest 1981) have also found to correlate well with egg-to-fry mortality. Including an effect for female size, expressed through the depth to which they buried their eggs, worsened model predictions of egg-to-fry mortality. Apparently, female size was not an important correlate of egg-to-fry mortality in Carnation Creek, in contrast with the findings of van den Berghe and Gross (1984).

Comparison of observed and predicted mean lengths and length frequency distributions of emergent fry also indicated that the progeny of large females enjoyed no consistent survival advantage over the progeny of small females. In fact, in the two instances in which observed fry sizes were substantially different from predicted (1977, 1983), the observed fry were smaller. These two years were also years of extreme high and low discharge, circumstances that might be expected to favour the offspring of large females if they were obtaining the "best" nest sites. In one year (1974), the fry were significantly larger than expected. The apparent advantage accruing to progeny of large females in this year was not associated with any of the factors that we would expect to favour the offspring of large females. Peak discharge (and hence scour) was moderate in 1974 (Table 2), gravel quality was above average (Table 2), and the abundance and size of females were both average (Table 1).

Contrary to fairly compelling evidence in the literature (Ricker 1976; Bilton et al. 1982), large smolts from Carnation Creek apparently did not consistently experience better marine survival than small smolts. In fact, in three of the four instances we examined, small smolts experienced a survival advantage over large smolts from the same brood year. When we compared the survival of different age classes of smolt that migrated to sea in the same year, there was no significant difference across all 12 years of data, although the range of sizes reprsented by the two age classes was large. Thus, if large female parents produced large smolts, these smolts would not necessarily experience a marine survival advantage. Furthermore, in Carnation Creek, smolt size and returning adult size were not correlated. Consequently, it is not obvious that size-dependent marine survival of smolts has anything directly to do with the reproductive success of different sized females.

It appears, therefore, that in Carnation Creek there is no consistent advantage to being a large female. At some times large size may even be a disadvantage. These observations are inconsistent with an hypothesis of size optimization but are consistent with an hypothesis that size variation among females represents a mixed ESS. We consider these observations to be a sufficient demonstration of the first requirement of an ESS, that large and small females have equal fitness. With regard to the second requirement of the mixed ESS hypothesis, that selection must be frequency dependent, we note that large and small females are likely to adopt different strategies in selecting spawning sites. Large females will occupy and defend high-flow sites with well-stored clean gravel, sites traditionally considered most suitable for spawning and incubation, but also most susceptible to disturbance by scour. Small females are likely to spawn in sites with lower flows and finer gravel, either through choice or because they are unable to compete with large females for the higher flow sites. These low-flow sites are traditionally considered poorer for spawning and incubation but the smaller eggs and fry of small females are well suited to such sites (van den Burghe 1984). Low-flow sites are also less susceptible to scour. Frequency-dependent selection seems to us to be a necessary consequence of these different spawning strategies. Certainly it seems inevitable that a small female producing small eggs could successfully invade a population of large females laying large eggs and vice versa (cf., Maynard Smith 1982).

If we assume that the mixture of sizes that will be evolutionarily stable in any stream is determined primarily by competition for nest sites, gravel quality, and scour during incubation, and that female size and egg size are positively correlated, then we can make a prediction about average female size and variance in size under different combinations of these factors. The necessary conditions are that competition favours large female size, as does heavy scour during incubation, whereas poor gravel quality favours small female size. Good gravel quality, low competition, and shallow scour are presumably relatively neutral with regard to female size, although shallow scour is likely to be associated with poor subgravel percolation, which would favour small females. Predicting the effects of these factors on variance in size is more difficult, but the assumption we make is that variance will be large when factors favouring large and small size are in conflict and variance will be small when factors favouring one size reinforce each other. Qualitative predictions of female size and variance under these conditions are shown in the following table (size to the left of the diagonal, variance to the right; L = large, M = moderate, S = small):

Gravel quality:	Good		Poor	
Competition:	High	Low	High	Low
Deep scour	L/S	L/L	M/S	M/M
Shallow scour	L/M	M/L	M/M	S/S

An important question thaı arises from our assertion that there is frequency-dependent selection for female size is what prevents large females laying small eggs from invading a small-bodied population? Our model predicts that small-bodied females should be the norm in streams with shallow scour, poor gravel, and little competiton. A large female laying small eggs would have a considerable fecundity advantage over small females in such a population and, thus, should be able to invade. We argue that such invasion would not occur because females

do not choose the depth to which they bury their eggs. Rather, nest depth is a result of the interplay of female digging power (as determined by her size), the composition of the spawning gravel (particle size, degree of cementing, etc.), and the hydraulic conditions around the nest site. With all else constant, nest depth should always be an increasing function of female size. Thus, a large female laying small eggs in a river system with poor subgravel percolation is likely to deposit her eggs too deep in the substrate to ensure their proper irrigation during incubation. Such a female would be at a disadvantage relative to small females in the same river, despite her greater fecundity.

Any test of the predictions of our model will require an extensive data set, since individual populations may not conform for a variety of reasons. We believe that the model is statistically falsifiable, however, and if corroborated would provide the basis for a quantitative model of coho size and variance in size under different stream habitat conditions.

Acknowledgments

We thank the various participants in the Carnation Creek project who permitted us to refer to their unpublished data. We also thank Gordon Hartman, Jake Schweigert, Colin Levings, and Bruce Leaman for critical comments on a draft of the paper.

References

ALLEN, G. H. 1958. Notes on the fecundity of silver salmon. Prog. Fish-Cult. 20: 163–169.

ANDERSEN, B. C. 1983. Fish populations of Carnation Creek and other Barkley Sound streams 1970–1980. Can. Data Rep. Fish. Aquat. Sci. 415: 267 p.

1984. Fish populations of Carnation Creek and other Barkley Sound streams 1981–1982. Can. Data Rep. Fish. Aquat. Sci. 435: 63 p.

ARGUE, A. W., AND S. R. HEIZER. 1974. Distribution maps and tables for tag recoveries from 1963–1969 coho and chinook taggings in British Columbia. Environ. Can. Fish. Mar. Serv. Data Rec. Ser. No. TAC/D-74-1: 309 p.

BEACHAM, T. D. 1982. Fecundity of coho salmon (*Oncorhynchus kisutch*) and chum salmon (*O. keta*) in the northeast Pacific Ocean. Can. J. Zool. 60: 1463–1469.

BILTON, H. T. 1984. Returns of chinook salmon in relation to juvenile size at release. Can. Tech. Rep. Fish. Aquat. Sci. 1245: 33 p.

BILTON, H. T., D. F. ALDERDICE, AND J. T. SCHNUTE. 1982. Influence of time and size at release of juvenile coho salmon (*Oncorhynchus kisutch*) on returns at maturity. Can. J. Fish. Aquat. Sci. 39: 426–447.

CLUTTER, R. I., AND L. E. WHITESEL. 1956. Collection and interpretation of sockeye salmon scales. Bull. Int. Pac. Salmon Fish Comm. 9: 1–159.

DRYBURGH, J. G. 1982. Carnation Creek logging and silvicultural treatment program, p. 36–43. *In* G. F. Hartman [ed.] Proceedings of the Carnation Creek workshop, a ten year review. Pacific Biological Station, Nanaimo, B.C.

FOWLER, L. G. 1972. Growth and mortality of fingerling chinook salmon as affected by egg size. Prog. Fish-Cult. 34: 66–69.

GILBERT, N. E. 1973. Biometrical interpretation. Claredon Press, Oxford, U.K.

GROSS, M. 1984. Sunfish, salmon and the evolution of alternative reproductive strategies and tactics in salmon, p. 55–75. *In* C. W. Potts and R. J. Wooton [ed.] Fish reproduction strategies in tactics. Academic Press, London, England.

1985. Disruptive selection for alternative life histories in salmon. Nature (Lond.) 313: 47–48.

HARTMAN, G. F. 1969. Reproductive biology of the Gerrard stock rainbow trout, p. 53–67. *In* T. G. Northcote [ed.] Symposium on salmon and trout in streams. H. R. MacMillan Lectures in Fisheries, University of British Columbia, Vancouver, B.C.

HARTMAN, G. F. [ED.]. 1982. Proceedings of the Carnation Creek workshop, a ten year review. (Available from Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, B.C. V9R 5K6, Canada)

HARTMAN, G. F., B. C. ANDERSEN, AND J. C. SCRIVENER. 1982. Seaward movement of coho salmon (*Oncorhynchus kisutch*) fry in Carnation Creek, an unstable coastal stream in British Columbia. Can. J. Fish. Aquat. Sci. 39: 588–597.

HEALEY, M. C. 1986. Optimum size and age at maturity in Pacific salmon and effects of size-selective fisheries, p. 39–52. *In* D. J. Meerburg [ed.] Salmonid age at maturity. Can. Spec. Publ. Fish. Aquat. Sci. 89: 118 p.

HEALEY, M. C., AND W. R. HEARD. 1984. Inter- and intrapopulation variation in the fecundity of chinook salmon (*Oncorhynchus tshawytscha*) and its relevance to life history theory. Can. J. Fish. Aquat. Sci. 41: 476–483.

1985. Reply to Hankin and McKelvie. Can. J. Fish. Aquat. Sci. 42: 394–395.

HOLTBY, L. B., G. F. HARTMAN, AND J. C. SCRIVENER. 1984. Stream indexing from the perspective of the Carnation Creek experience, p. 87–112. *In* P. E. K. Symons and M. Waldichuk [ed.] Proceedings of the workshop: stream indexing for salmon escapement estimation. Can. Tech. Rep. Fish. Aquat. Sci. 1326: 258 p.

LOTSPEICH, F. B., AND F. H. EVEREST. 1981. A new method for reporting and interpreting textural composition of spawning gravel. U.S. Dep. Agric. For. Serv. Res. Note P.N.W. 369: 1–11.

MACKAY, T. F. C. 1980. Genetic variance, fitness, and homeostasis in varying environments: an experimental check of the theory. Evolution 34: 1219–1222.

MAYNARD SMITH, J. 1982. Evolution and the theory of games. Cambridge University Press, Cambridge, England. 224 p.

MCNEIL, W. J. 1966. Effect of the spawning bed environment on reproduction of pink and chum salmon. U.S. Fish. Wildl. Serv. Fish. Bull. 64: 495–523.

MORING, J. R. 1975. The Alsea watershed study: effects of logging on the aquatic resources of three headwater streams of the Alsea River, Oregon. Part II — Changes in environmental conditions. Oreg. Dep. Fish. Wildl. Corvallis, Fish. Res. Pap. 9: 1–39.

PALOHEIMO, J. E., AND P. F. ELSON. 1974. Reduction of Atlantic salmon (*Salmo salar*) catches in Canada attributed to the Greenland fishery. J. Fish. Res. Board Can. 31: 1467–1480.

PHILLIPS, R. W. 1971. Effects of sediment on the gravel environment and fish production, p. 64–74. *In* J. T. Krygier and J. O. Hall [ed.] Proceedings of a symposium: forest land uses and stream environment. Oregon State University, Corvallis, OR.

PHILLIPS, R. W., L. L. LUTZ, E. W. CLAIRE, AND J. R. MORING. 1975. Some effects of gravel mixtures on emergence of coho salmon and steelhead trout fry. Trans. Am. Fish. Soc. 105: 461–465.

PLATTS, W. S., W. F. MEGAHAN, AND G. W. MINSHALL. 1983. Methods for evaluating stream, riparian, and biotic conditions. Gen. Tech. Rep. INT-138: 1–70. U.S. Department of Agriculture, Forest Service, Intermountain Forest and Range Experimental Station, Ogden, UT.

RICKER, W. E. 1976. Review of the rate of growth and mortality of Pacific salmon in salt water, and noncatch mortality caused by fishing. J. Fish. Res. Board Can. 33: 1483–1524.

1980a. Changes in the age and size of chum salmon (*Oncorhynchus keta*). Can. Tech. Rep. Fish. Aquat. Sci. 930: 99 p.

1980b. Causes of the decrease in age and size of chinook salmon (*Oncorhynchus tshawytscha*). Can. Tech. Rep. Fish. Aquat. Sci. 944: 25 p.

1981. Changes in the average size and average age of Pacific salmon. Can. J. Fish. Aquat. Sci. 38: 1636–1656.

1982. Size and age of British Columbia sockeye salmon (*Oncorhynchus nerka*) in relation to environmental factors and the fishery. Can. Tech. Rep. Fish. Aquat. Sci. 1115: 117 p.

RICKER, W. E., H. T. BILTON, AND K. V. ARO. 1978. Causes of the decrease in size of pink salmon (*Oncorhynchus gorbuscha*). Can. Tech. Rep. Fish. Aquat. Sci. 820: 93 p.

RICKER, W. E., AND W. P. WICKETT. 1980. Causes of the decrease in size of coho salmon (*Oncorhynchus kisutch*). Can. Tech. Rep. Fish. Aquat. Sci. 971: 63 p.

ROFF, D. A. 1981. On being the right size. Am. Nat. 118: 405–422.

ROUNSEFELL, G. A. 1957. Fecundity of North American Salmonidae. U.S. Fish. Wildl. Serv. Fish. Bull. 57: 450–468.

RYAN, P. 1970. Design and operation of an in situ frozen core gravel sampler. Can. Dep. Fish. For. Tech. Rep. 1970-12: 1–8.

SCRIVENER, J. C., AND M. J. BROWNLEE. 1982. An analysis of the Carnation Creek gravel quality data 1973–1981, p. 154–173. *In* G. F. Hartman [ed.] Proceedings of the Carnation Creek workshop, a ten year review. Pacific Biological Station, Nanaimo, B.C.

SEBER, G. A. F., AND E. D. LECREN. 1967. Estimating population parameters from catches large relative to the population. J. Anim. Ecol. 36:

631–643.

STEARNS, S. C., AND R. E. CRANDALL. 1984. Plasticity for age and size at sexual maturity: a life history response to unavoidable stress, p. 13–33. *In* C. W. Potts and R. J. Wooten [ed.] Fish reproduction strategies and tactics. Academic Press, London, England.

VAN DEN BERGHE, E. P. 1984. Natural selection and reproductive success of female coho salmon (*Oncorhynchus kisutch*): a study in female competition. M.Sc. thesis, Simon Fraser University, Burnaby, B.C. 71 p.

VAN DEN BERGHE, E. P., AND M. R. GROSS. 1984. Female size and nest depth in coho salmon (*Oncorhynchus kisutch*). Can. J. Fish. Aquat. Sci. 41: 204–206.

VAN VALEN, L. 1965. Morphological variation and width of the ecological niche. Am. Nat. 94: 377–390.

Photosynthetic Characteristics of Lotic Periphyton

Stephen Jasper[1] and Max L. Bothwell[2]

National Water Research Institute, Pacific and Yukon Region, Inland Waters Directorate, Environment Canada, 4160 Marine Drive, West Vancouver, B.C. V7V 1N6

Jasper, S., and M. L. Bothwell. 1986. Photosynthetic characteristics of lotic periphyton. Can. J. Fish. Aquat. Sci. 43: 1960–1969.

Periphyton communities, grown in continuous-flow troughs, were suspended in filtered river water and placed in a photosynthesis–light incubator similar to those used for phytoplankton. A three-parameter equation was fitted to the incubator data. Seasonally the initial slope, α, ranged from 12.1 to 53.6 $\mu g\ C \cdot (mg\ Chl\ a)^{-1} \cdot h^{-1} \cdot (\mu Einst. \cdot m^{-2} \cdot s^{-1})^{-1}$; the maximum photosynthetic rate, P^B_{max}, varied from 1.16 to 5.49 $mg\ C \cdot (mg\ Chl\ a)^{-1} \cdot h^{-1}$; and the inhibition parameter, I_b, ranged from 1500 to 6900 $\mu Einst. \cdot m^{-2} \cdot s^{-1}$. These values are similar to those reported for other periphyton and phytoplankton. P^B_{max} strongly correlated with light history, whereas inhibition was inversely related to water temperature. α did not correlate with either irradiance or temperature but with changes in average cell size. Seasonal variations in the light level optimal for photosynthesis, I_m, suggested a period in late winter and early spring for potentially serious inhibition of photosynthesis. These Chl *a* normalized photosynthetic parameters were unaffected by changes in concentration of the growth-limiting nutrient, phosphorus.

Des communautés de périphyton, élevées dans des bassins à écoulement continu, ont été mises en suspension dans de l'eau de rivière filtrée et placées dans un incubateur éclairé à photosynthèse semblable à ceux qui sont utilisés pour le phytoplancton. Une équation à trois paramètres a été ajustée en fonction des caractéristiques de l'incubateur. De façon saisonnière, la pente initiale, α, allait de 12,1 à 53,6 $\mu g\ C \cdot (mg\ Chl\ a)^{-1} \cdot h^{-1} \cdot (\mu Einst \cdot m^{-2} \cdot s^{-1})^{-1}$; le maximum de photosynthèse, P^B_{max}, variait de 1,16 à 5,49 $mg\ C \cdot (mg\ Chl\ a)^{-1} \cdot h^{-1}$; et le paramètre d'inhibition, I_b, allait de 1 500 à 6 900 $\mu Einst \cdot m^{-2} \cdot s^{-1}$. Ces chiffres sont similaires à ceux qui ont été rapportés pour d'autres types de périphyton et de phytoplancton. P^B_{max} présentait une forte corrélation avec l'éclairement, tandis que l'inhibition était inversement reliée à la température de l'eau. α n'était pas en corrélation avec l'éclairement ni avec la température mais avec des changements dans la taille moyenne des cellules. Les variations saisonnières de l'éclairement optimal pour la photosynthèse, I_m, indiquent qu'il existe une période, à la fin de l'hiver et au début du printemps, où la photosynthèse peut être fortement inhibée. Ces paramètres photosynthétiques normalisés en fonction de la Chl *a* n'étaient pas affectés par des changements dans la concentration du phosphore, qui constitue le nutriment limitant de la croissance.

Received January 31, 1986
Accepted June 27, 1986
(J8667)

Reçu le 31 janvier 1986
Accepté le 27 juin 1986

In contrast with phytoplankton, the existing knowledge about periphyton photosynthesis is scarce. This is understandable, since natural periphyton communities are inherently more difficult to study. For example, substratum heterogeneity is a serious problem that often leads to high variability in experimental data (Wetzel 1965; Schindler et al. 1973; Jones and Adams 1982; Robinson 1983), and the placing of periphyton in any kind of enclosure for photosynthesis measurements affects the normal water flow patterns and requires some attempt at mixing to try to simulate in situ flow conditions. This is particularly important for lotic periphyton.

Methods have been developed to overcome some of these problems. Various incubation chambers have been devised to enclose periphyton samples. Some provide for a certain amount of mixing within the chamber (McIntire et al. 1964; Thomas and O'Connell 1966; Marker 1976; Rodgers et al. 1978), and in a few the light intensity to which the sample is exposed can be varied (McIntire et al. 1964; Pfeifer and McDiffett 1975; Jones and Adams 1982; Turner et al. 1983). Artificial substrata have been used (see review in Wetzel 1964; Loeb 1981; Turner et al. 1983; Robinson 1983) to decrease heterogeneity and provide substantially improved replicate variability and ease of sample manipulation. In addition, recirculating or continuous-flow troughs have been employed with both natural (McIntire et al. 1964; McIntire and Phinney 1965) and artificial (Bothwell 1983, 1985; Bothwell and Jasper 1983; Horner et al. 1983) substrata to permit colonization and growth of periphyton under controlled conditions. However, despite these advances there is still a relatively limited knowledge of lotic periphyton photosynthesis.

In this paper we describe the results of a series of photosynthesis experiments using periphyton colonized and grown on styrofoam substrata in continuous-flow troughs. Suspension of periphyton in filtered river water enabled the use of a light incubator similar to those commonly used for phytoplankton. Photosynthesis–light ($P(I)$) data were obtained for communities grown at several different levels of phosphorus enrichment at different times of the year. These photosynthesis studies were part of a larger investigation into the influence of

[1]Present address: 979 Belgrave Ave., North Vancouver, B.C. V7R 1Z3.

[2]Author to whom reprint requests should be addressed. Present address: National Hydrology Research Center, 11 Innovation Blvd., Saskatoon, Sask. S7N 3H5.

phosphorus on the growth rate and physiology of lotic periphyton (Bothwell and Jasper, unpubl. data).

Methods

Periphyton Colonization and Sampling

Periphyton for these experiments was obtained from the experimental troughs research apparatus situated adjacent to the South Thompson River at Chase, B.C. (Jasper et al. 1984; Bothwell and Jasper, unpubl. data). Details of this continuous-flow trough methodology have been reported previously (Bothwell 1983). River water was pumped to a set of Plexiglas troughs each containing styrofoam-DB substratum. Periphyton was allowed to accumulate for 2–4 wk under natural light conditions and a constant flow velocity of 50 $cm \cdot s^{-1}$. Covers constructed of UV-resistant Plexiglas (UF1-type, Rohm and Haas, Toronto) protected the troughs and blocked any possible UV light from reaching the periphyton. In each experiment different amounts of orthophosphate were added continuously to the inflowing water in each of the troughs. This provided a range of added phosphorus concentrations from 0 to 5 μg $P \cdot L^{-1}$. Otherwise, conditions between the troughs were kept constant. At the end of an experiment the concentration of algae was typically around 5 mg Chl $a \cdot m^{-2}$ in the trough without added phosphorus and between 50 and 100 mg Chl $a \cdot m^{-2}$ in the troughs treated with 5 μg $P \cdot L^{-1}$. Ten experiments were run from April 1984 to March 1985.

For photosynthesis measurements, periphyton samples were collected by removing sections of styrofoam from the troughs and shaking the attached material into wide-mouth 2-L bottles containing filtered river water. The suspensions were agitated vigorously to break up algal clumps and then screened through 550-μm Nitex mesh. Taxonomic examination confirmed that these suspensions were representative of the total algal community that had accumulated on the styrofoam.

The concentration of the periphyton suspension had a pronounced effect on the shape of the $P(I)$ curve. Higher concentrations (>50 μg Chl $a \cdot L^{-1}$) tended to show a reduction in light inhibition, suggesting self-shading within the incubation bottle and a resultant decrease in the light available to the suspensions. For this reason all of the suspensions were kept between 5 and 10 μg Chl $a \cdot L^{-1}$.

Water Chemistry and Surface Irradiance Measurements

Total inorganic carbon was determined by gas chromatography (Stainton 1973); soluble reactive phosphorus (SRP) and dissolved nitrogen species were obtained using methods outlined in Bothwell (1985). Subsamples from the periphyton suspensions were removed for other analyses. Chl *a* was determined by filtration, extraction with 90% acetone using a Polytron grinder, and subsequent fluorometry (Holm-Hansen et al. 1965). Species composition and volumes were estimated from Lugol's-fixed subsamples as outlined in Bothwell (1985).

The surface irradiance was monitored continuously using a PAR-quantum light sensor and printing integrator (LI-COR, Nebraska). Data was obtained at 1-h intervals and converted to quantum units (microEinsteins per square metre per second). Reflection loss at the air–water interface and loss due to the plastic cover over the trough were estimated jointly by placing the sensor underneath an inverted trough cover and diverting the water flow over the top. Measurements were repeated several times at different sun angles and cloud covers.

Photosynthesis–Light Curves

To determine the photosynthesis versus light ($P(I)$) relationships the periphyton suspensions were placed in an incubator similar to those used for phytoplankton work (Fee 1969; Gargas et al. 1976; Jasper et al. 1983). Illumination was provided by a 1000-W metal-halide lamp and reflector (GTE Sylvania) with a spectral distribution from 400 to 650 nm and a peak intensity around 510 nm. As a precaution a screen of 3-mm-thick UF1 Plexiglas (Rohm and Haas, Toronto) was used to block any possible UV light from reaching the suspensions. Attenuation of the light to each of four incubator chambers was accomplished using neutral density screens; the levels in each chamber were determined using an underwater PAR-quantum sensor (LI-COR, Nebraska). Measurements were repeated several times throughout the year and whenever the neutral density screens were changed.

Light levels (microEinsteins per square metre per second) in the four chambers of the incubator were typically 1923, 701, 261, and 96 during the summer months but were attenuated during other times of the year and reached minimum levels of 889, 323, 113, and 39 in the winter. These light levels are higher than those normally reported for other incubators, especially those used to investigate phytoplankton populations. The intention was to simulate the maximum seasonal in situ light conditions that could be expected in the troughs and thus to examine the photoinhibition part of the $P(I)$ curve. Because these periphyton populations are grown in shallow water (≈1 cm depth) with no shading, there is very little attenuation of natural light.

Samples were siphoned into prerinsed 60-mL glass bottles and injected with 1 mL of a stock $NaH^{14}CO_3$ solution. This stock was freshly prepared for each experiment by filtering an ampoule of isotope (New England Nuclear) through a 0.45-μm Millipore filter and making up to a concentration of 0.4 $MBq \cdot mL^{-1}$ using filter–sterilized, pH 8.5, deionized water. Triplicate bottles for each sample were then immediately placed in the incubator in each of the four light chambers while a fifth set was used as a "zero-time" blank (Morris et al. 1971). Incubations were carried out within 0.5°C of ambient river temperatures.

An experiment was conducted to determine if any changes occur in the magnitude or shape of the $P(I)$ curve with incubation time. Except for the short 25-min incubation, there were no significant changes in productivities, with time, at any of the light levels (Fig. 1). Routine incubations were completed within 2–3 h.

Following incubation, samples were filtered for determination of particulate ^{14}C activity. The entire contents of each bottle were filtered (<25 cm Hg vacuum) through a 47-mm Millipore 0.45-μm filter and washed with 10 mL of filtered river water to remove inorganic ^{14}C activity remaining in the filter matrix. Each filter was placed in a scintillation vial containing 10 mL of Aquasol-2 (New England Nuclear). After 1 h, which was an adequate time to allow filter dissolution and release of labelled material, the samples were counted in a LKB Rack-Beta scintillation counter. Internal standardization with ^{14}C-labelled toluene was used to determine quenching. For routine experiments, incubations were performed in triplicate with the coefficient of variation usually <10%.

On three separate dates, additional incubations were performed using undisturbed periphyton communities. For these trials, small sections (1 × 8 cm) of the styrofoam substratum

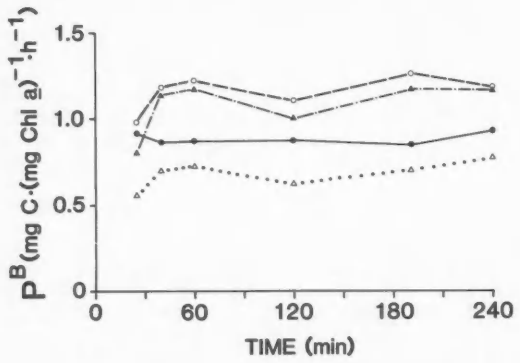


FIG. 1. Productivity of a lotic periphyton suspension incubated for six different time intervals at four light levels: 1923 (●), 701 (○), 261 (▲), and 96 μEinst.·m^{-2}·s^{-1} (△), April 11, 1984. Error bars have been omitted for clarity (average SD of triplicate incubations = 7.4%).

with attached periphyton were carefully removed from the troughs and placed in the incubation bottles previously filled with ^{14}C-labelled filtered river water. Notched rubber bungs were used to hold the styrofoam slices in place and orient them to the light source. At the end of the incubation, each slice was placed in 100 mL of deionized water and shaken vigorously. After screening through 550-μm Nitex mesh, aliquots were removed to determine particulate ^{14}C activity and Chl *a* concentration.

Curve Fitting

Several mathematical representations of the photosynthesis–light response have been used over the years. Most of them describe that part of the $P(I)$ curve up to and including light saturation and have been applied to phytoplankton (Talling 1957; Jassby and Platt 1976; Platt et al. 1977) and periphyton (Jones and Adams 1982; Turner et al. 1983; Carpenter 1985). In these cases the subsaturation part of the $P(I)$ curve is represented either by a half-saturation parameter, I_k, or by the initial slope, α. The latter term has more inherent value because it is independent of the maximum rate of photosynthesis, P_{max}, and consequently has been adopted more frequently. However, since inhibition was a common occurrence in our data sets, all of these equations were deemed inadequate. Some representations of the full $P(I)$ curve, including inhibition, utilize either four parameters (Vollenweider 1965) or an equation that does not have independent slopes on either side of P_{max} (Steele 1962).

A more recent formula suggested by Platt et al. (1980) has the advantage of only one additional parameter, β, to characterize inhibition. Photosynthesis, normalized to Chl *a*, is given by

$$P^B = P^B_s \cdot (1 - e^{-a}) \cdot e^{-b}$$

where $a = \alpha \cdot I / P^B_s$ and $b = \beta \cdot I / P^B_s$. Figure 2 shows the general form of this equation together with some of the derived parameters. P^B_s represents the maximum potential photosynthesis that would occur if there were no photoinhibition. Its relationship to the actual maximum rate P^B_{max}, again normalized to Chl *a*, is represented by

$$P^B_{max} = P^B_s \cdot \left(\frac{\alpha}{\alpha + \beta}\right) \cdot \left(\frac{\beta}{\alpha + \beta}\right)^{\beta/\alpha}$$

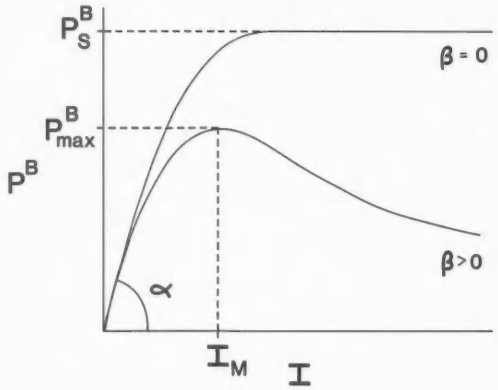


FIG. 2. General form of the $P(I)$ equation with derived parameters (see text). Modified from Platt et al. (1980).

Note that when $\beta = 0$, $P^B_{max} = P^B_s$. I_m, the light level optimal for photosynthesis, corresponding to P^B_{max}, is found from

$$I_m = \frac{P^B_s}{\alpha} \cdot \log_e\left(\frac{\alpha + \beta}{\beta}\right).$$

The equation was fitted to the data by nonlinear regression using the method of partial derivatives and forcing the curve through the origin (Platt et al. 1980). This may result in errors estimating α as ^{14}C uptake changes from representing "net" to "gross" photosynthesis at low light levels (Turner et al. 1983). However, because dark respiration rates are usually small (Platt and Jassby 1976), these errors in α are likely to be minor.

Because the magnitude of β is dependent on P^B_s, an additional index was obtained, $I_b = P^B_s/\beta$, which corresponds to the light intensity, above the optimal, where $P^B = 0.37P^B_s$. This index plus other frequently used symbols in this paper are given in Table 1.

Results and Discussion

Experimental Conditions

Incubation temperatures ranged from 0.8°C in January to 17.9°C in August (Table 2). Surface irradiance (integrated over the day) varied from lows in November to highs in June and July, around 2 and 55 Einst.·m^{-2}·d^{-1}, respectively. Total loss of light by reflection and absorption by the trough covers and water flow averaged 12%. For the purposes of comparison with productivity parameters, the average daily irradiance levels immediately prior to each incubation run were calculated for 3-, 5-, and 10-d periods (I_3, I_5, I_{10}, respectively, Table 2). Most of the time these three values were similar. The one exception was in August during a period of rapidly changing light levels.

Nutrient levels in the inflowing river water were generally low throughout the period. SRP was usually between 1 and 3 μg P·L^{-1}, and phosphorus is known to be the nutrient limiting algal growth in this river (Bothwell 1985). Dissolved inorganic nitrogen ($NO_3 + NO_2$) concentrations were usually between 60 and 100 μg N·L^{-1}, except for the period between August and October when levels dropped below 20 μg N·L^{-1}.

The algal community attached to the substratum at the end of each experiment was composed almost exclusively of

TABLE 1. List of frequently used symbols, their explanation, and units.

Symbol	Explanation	Units
P^B	Photosynthetic rate	mg C·(mg Chl *a*)$^{-1}$·h^{-1}
P^B_s	Maximum potential photosynthetic rate that would occur with no inhibition	mg C·(mg Chl *a*)$^{-1}$·h^{-1}
P^B_{max}	Maximum observed photosynthetic rate when inhibition present	mg C·(mg Chl *a*)$^{-1}$·h^{-1}
α	Initial slope of the photosynthesis–light curve	μg C·(mg Chl *a*)$^{-1}$·h^{-1}·(μEinst.·m^{-2}·s^{-1})$^{-1}$
β	Inhibition parameter of the photosynthesis–light curve	μg C·(mg Chl *a*)$^{-1}$·h^{-1}·(μEinst.·m^{-2}·s^{-1})$^{-1}$
I_b	Inhibition index independent of P^B_s: $I_b = P^B_s/\beta$	μEinst.·m^{-2}·s^{-1}
I_m	Light level optimal for photosynthesis	μEinst.·m^{-2}·s^{-1}
I_3	Average daily surface irradiance for the previous 3 d	Einst.·m^{-2}·d^{-1}
I_5	Average daily surface irradiance for the previous 5 d	Einst.·m^{-2}·d^{-1}
I_{10}	Average daily surface irradiance for the previous 10 d	Einst.·m^{-2}·d^{-1}
T	Incubation temperature	°C

TABLE 2. Environmental parameters for the periphyton incubation experiments. T = temperature (°C); I_3, I_5, and I_{10} = average daily irradiance (Einst.·m^{-2}·d^{-1}) for the previous 3, 5, and 10 d, respectively. Also shown are the two most common algal species and their percent by volume (averaged for all treatments). nd = not determined.

Date	T	I_3	I_5	I_{10}	Dominant algal species	%
April 11, 1984	5.1	22.6	19.7	19.7[a]	nd	
May 2, 1984	6.4	22.7	27.1	28.6	*Fragilaria crotonensis*	23
					Hannaea arcus	17
May 29, 1984	9.1	34.2	35.8	30.1	nd	
July 4, 1984	12.9	34.2	33.3	31.5	*Fragilaria crotonensis*	38
					Hannaea arcus	20
August 29, 1984	17.9	16.5	23.8	29.3	*Tabellaria fenestrata*	54
					Synedra ulna	13
September 28, 1984	14.3	22.6	21.6	18.3	*Tabellaria fenestrata*	55
					Fragilaria crotonensis	13
November 6, 1984	7.4	8.2	6.1	7.9	*Tabellaria fenestrata*	47
					Fragilaria crotonensis	7
January 22, 1985	0.8	4.5	3.9	4.0	*Tabellaria fenestrata*	45
					Fragilaria crotonensis	12
February 27, 1985	0.9	13.4	11.6	11.0	*Tabellaria fenestrata*	21
					Hannaea arcus	23
March 26, 1985	1.9	15.5	14.6	18.5	*Tabellaria fenestrata*	27
					Diatoma tenue	25

[a]8-d average only.

diatoms. Depending on the time of year, between 60 and 95% of the community cell volume was composed of six species: *Tabellaria fenestrata*, *Fragilaria crotonensis*, *F. vaucheriae*, *Synedra* sp., *Hannaea arcus*, and *Diatoma tenue*. Seasonal shifts occurred in the relative magnitude of these species (Table 2); *Fragilaria* spp. and *H. arcus* dominated until July, while *T. fenestrata* dominated from August through January.

General Methodology

The importance of current and physical mixing on lotic periphyton respiration and photosynthesis has been well documented (Whitford and Schumacher 1961; McIntire 1968; Pfeifer and McDiffett 1975; Rodgers et al. 1978). For this reason, incubation chambers that enclose attached algae have to be designed with some kind of internal mixing or forced circulation to try to simulate the flow conditions of the sample's habitat. In most cases it is difficult to ascertain whether natural conditions are indeed being met and that measured photosynthetic rates are truly representative. For fast-flowing habitats the use of incubation chambers is especially difficult (Pfeifer and McDiffett 1975). Periphyton suspensions, however, tend to alleviate this problem by ensuring intimate contact between cells and medium, and the use of an incubator with bottle rotation provides constant movement of this suspended material within the medium. This simulates the nutrient diffusion conditions that would occur with fast water flows. Although suspensions are used routinely for phytoplankton and have been adopted, by necessity, for epipelic algae (Hunding 1971; Colijn and van Buurt 1975; Bott and Ritter 1981), they have not been used for lotic periphyton.

TABLE 3. Comparison between photosynthetic productivity, P^B (mg C·(mg Chl a)$^{-1}$·h^{-1}), of intact periphyton communities and periphyton suspensions (in parentheses). The relationship of intact to suspended samples is shown underneath as a percentage.

	July 5, 1984	September 27, 1984	November 6, 1984
Biomass[a] (mg Chl a·m^{-2})	30	16	65
Light chamber			
1 (highest light)	1.37 (4.27)	2.01 (3.01)	0.20 (1.08)
	32	67	19
2	1.89 (5.29)	1.90 (3.11)	0.25 (1.40)
	36	61	18
3	1.52 (4.86)	1.85 (2.34)	0.25 (1.48)
	31	79	17
4 (lowest light)	1.09 (2.40)	1.36 (0.92)	0.21 (0.86)
	45	148	24

[a]Periphyton biomass attached to styrofoam substratum prior to sampling.

TABLE 4. Two-way analysis of variance for photosynthetic parameters versus phosphorus treatment. Data are taken from four comparable treatments, 0, 0.2, 1, and 5 μg·L^{-1} of added P, for the 10 experiments (n = 40).

Parameter	F-value	Significance
P^B_{max}	1.32	NS
α	2.58	NS
I_b	0.60	NS
0.05 F[3,27] = 2.96		

A potential problem with periphyton suspensions, however, is that the physical structure of the community is disrupted. This could lead to enhanced mineral uptake rates as the diffusion gradients around cells, otherwise not in contact with overlying water, are artificially increased. In addition, light availability to individual cells may be increased as natural self-shading effects are diminished. These problems would tend to increase with periphyton mat thickness and complexity. For this reason we ran several experiments comparing $P(I)$ curves of periphyton suspensions with intact communities (Table 3). With one exception the suspensions always had higher rates than the attached samples and the differences between them increased with original mat thickness (represented by Chl a concentrations). The appearance of light limitation in the attached samples, similar to that in the suspensions, would tend to discount the hypothesis of altered light availability. Similarly, with a single exception, the productivities of attached periphyton as a percentage of suspensions were reasonably constant throughout the light gradient. This again would suggest that light conditions were not responsible for the differences in rates. A more likely explanation is one of differences in diffusion characteristics. Furthermore, although these experiments do not prove one way or the other whether the suspensions have enhanced rates compared with in situ communities, it seems more reasonable that the attached material within the incubation bottles was showing depressed rates. The original periphyton has been grown in water flow velocities around 50 cm·s^{-1}, conditions quite different from those encountered by the attached material within the bottles. These experiments, then, reinforce the emphasis of previous workers on the need to maintain adequate physical mixing conditions for lotic periphyton work.

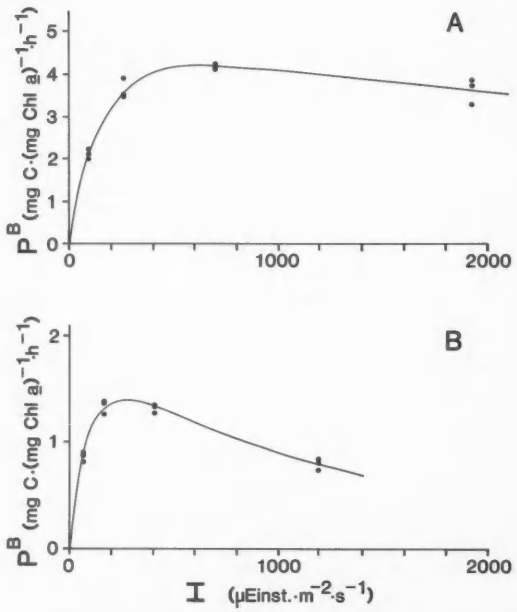


FIG. 3. Two typical lotic periphyton $P(I)$ data sets and fitted curves that represent changes in photosynthetic characteristics throughout the year: (A) July 4, 1984, 0 μg·L^{-1} added P, α = 29.6, P^B_{max} = 4.18, I_b = 8200, I_m = 620; (B) November 6, 1984, 0.2 μg·L^{-1} added P, α = 19.6, P^B_{max} = 1.40, I_b = 1460, I_m = 260 (units as in Table 1).

Photosynthesis–Light Curves

Despite large changes in specific growth rates and biomass of periphyton with phosphorus addition (Bothwell and Jasper, unpubl. data) there were no changes in Chl a normalized $P(I)$ curves. A two-way analysis of variance using data from the 0, 0.2, 1, and 5 μg P·L^{-1} additions (n = 40, Table 4) confirmed no significant differences in P^B_{max}, α, or I_b with phosphorus treatment. The average variation of P^B_{max} for the four treatments was 12.4% (±7.4%). For this reason the data obtained from all the treatments on any one date were averaged for the examination of seasonal trends (Table 5).

The curve for July (Fig. 3) represents the general features of many of the data sets during the summer period. Thus, we see

TABLE 5. Parameters (see Table 1 for units) of the photosynthesis–light curve for lotic periphyton. Data from all treatments[a] on each date have been averaged (SD in parentheses).

Date	α	P^B_{max}	P^B_s	β	I_b	I_m
April 11, 1984	12.1	1.48	1.77	0.47	3900	480
	(1.6)	(0.14)	(0.24)	(0.17)	(1000)	(40)
May 2, 1984	34.2	3.40	3.99	1.33	4000	410
	(7.6)	(0.30)	(0.50)	(0.83)	(2200)	(90)
May 29, 1984	43.6	5.49	7.25	2.63	3100	470
	(9.7)	(1.63)	(2.54)	(1.69)	(670)	(30)
July 4, 1984	39.6	5.45	6.46	1.58	4600	540
	(11.9)	(1.24)	(1.45)	(0.68)	(1800)	(50)
August 29, 1984	26.3	3.37	3.72	0.79	6900	510
	(5.8)	(0.55)	(0.78)	(0.61)	(4200)	(100)
September 28, 1984	19.1	2.72	3.27	0.83	4300	550
	(4.3)	(0.32)	(0.36)	(0.22)	(1200)	(60)
November 6, 1984	17.5	1.29	1.59	0.95	1800	270
	(3.3)	(0.26)	(0.32)	(0.30)	(500)	(20)
January 22, 1985	39.6	1.16	1.33	1.26	1500	130
	(6.8)	(0.09)	(0.14)	(0.65)	(1300)	(30)
February 27, 1985	48.6	2.06	2.47	1.94	1500	170
	(10.1)	(0.22)	(0.50)	(1.19)	(640)	(20)
March 26, 1985	53.6	1.99	2.25	1.37	1700	160
	(10.8)	(0.39)	(0.47)	(0.47)	(360)	(20)

[a] $n = 7$ for April 11, 1984, to September 28, 1984; $n = 4$ for November 6, 1984, to March 26, 1985.

TABLE 6. Simple correlation coefficients ($n = 10$) between periphyton photosynthetic parameters, incubation temperature, and previous irradiance averages (*$0.01 < p < 0.05$; **$p < 0.01$).

	P^B_{max}	I_b	T	I_3	I_5	I_{10}
α	0.276	−0.418	−0.435	0.054	0.066	0.057
P^B_{max}		0.489	0.556	0.875**	0.922**	0.852**
I_b			0.876**	0.473	0.632*	0.741*
T				0.470	0.587	0.630
I_3					0.955**	0.851**
I_5						0.953**

relatively high values for maximum photosynthesis, P^B_{max}; initial slope, α; the light level corresponding to P^B_{max}, I_m; and the inhibition parameter, I_b. In contrast, the curve for November represents the typical winter features when all of these parameters were lower.

Photosynthetic Parameters: P^B_{max}

We found seasonal changes in P^B_{max} from 1.16 to 5.49 mg C·(mg Chl *a*)$^{-1}$·h^{-1} (Table 5) which are within the range of values found elsewhere. Phytoplankton, for example, have been found to have levels between 0.5 and 30 (Platt and Jassby 1976; Harris 1980; Platt et al. 1980; Jasper et al. 1983) although typical levels, for marine phytoplankton, are between 1 and 10 (Platt and Subba Rao 1975). Periphyton studies have found values ranging from 0.2 to 5.7 (McIntire and Phinney 1965; Marker 1976; Bott and Ritter 1981; Jones and Adams 1982), although higher rates of 15.2 to 30.9 have been reported for tropical coral reef communities (Carpenter 1985).

P^B_{max} is a function of enzymatic rates during the dark reactions of photosynthesis. Thus, its dependence on temperature is expected from theory and has been well documented for natural populations of phytoplankton (Harris 1980; Falkowski 1981) and also for periphyton (Jones and Adams 1982). In addition, the high values found by Carpenter (1985) are in part due to the warm ambient temperatures (≈28°C) surrounding the tropical reef algae. Although our P^B_{max} values were generally high in the summer and low in winter, we did not find a significant correlation with temperature (Table 6; Fig. 4).

There was, however, a highly significant correlation of P^B_{max} with ambient light levels, represented by the previous 3-, 5-, or 10-d irradiance averages (Table 6; Fig. 4). Correlations of P^B_{max} with light have been found previously in algal cultures (Beadall and Morris 1976; Perry et al. 1981), and a correlation with long-term average irradiance was found in epiphytes (Jones and Adams 1982). The correlation was not found, however, with 3-d light averages in phytoplankton (Platt and Jassby 1976) or 1-, 3-, and 5-d light averages in epiphytes (Jones and Adams 1982). This dependence of P^B_{max} on light history could be due to light induction of enzymes responsible for the dark reactions of photosynthesis (Halldal 1970). Alternatively, because P^B_{max} is a ratio, light may be affecting the chlorophyll content per cell (Falkowski 1981; Perry et al. 1981). In either case our data indicate a very strong coupling between light history and P^B_{max}.

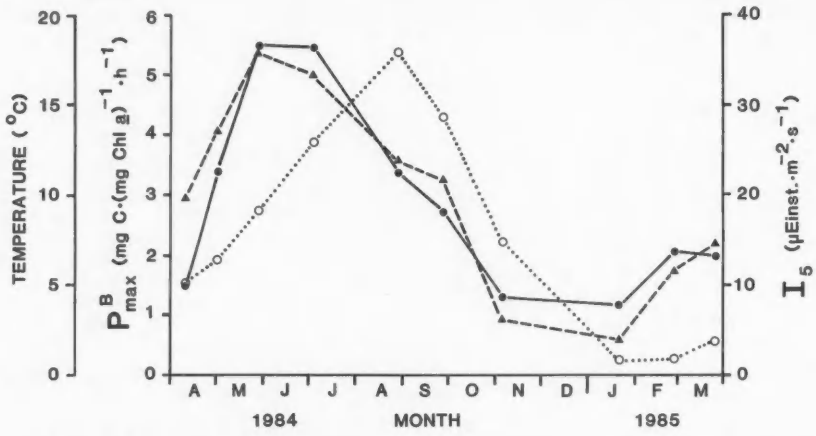


FIG. 4. Seasonal trends in P^B_{max} (●), temperature (○), and 5-d average irradiance, I_5 (▲).

The dependence of P^B_{max} on nutrients has been observed frequently in phytoplankton (Thomas 1969; Glooschenko et al. 1974; Falkowski 1981). In many cases, higher values are associated with increased seasonal availability of nutrients or with changes in the trophic status of the system. We were unable to find any such change in P^B_{max} with phosphorus addition despite the fact that growth rates of these periphyton are known to be phosphorus limited (Bothwell 1985; Bothwell and Jasper, unpubl. data). Because the conversion from photosynthesis per unit chlorophyll to growth rate requires the use of carbon to chlorophyll ratios, we determined this ratio several times using the method of Redalje and Laws (1981) but were unable to find significant differences with phosphorus treatment (unpublished data). A further possibility is that increases in growth rate with phosphorus treatment represent decreases in the loss rates of carbon due to excretion or respiration. Neither of these processes was examined.

Initial Slope, α

The initial slope of the $P(I)$ curve, or photosynthetic efficiency normalized for Chl *a*, α, varied from 12.1 to 53.6 (μg C·(mg Chl *a*)$^{-1}$·h^{-1}·(μEinst.·m^{-2}·s^{-1})$^{-1}$) throughout the year (Table 5). These values are within the range for cultures and natural populations of phytoplankton (Platt and Jassby 1976; Platt et al. 1980; Perry et al. 1981; Harding et al. 1982) and for periphyton (Jones and Adams 1982; Carpenter 1985). Despite this seasonal variation, we did not find any correlation with temperature or light history (Table 6). Because α represents the photochemical reactions of photosynthesis, its independence from temperature is not surprising. However, many studies have shown changes in the initial slope as individual cells or communities respond to their light environment (the classical "sun" and "shade" populations). Several reasons have been advanced for this adaptation including changes in Chl *a* content per cell by the change in size and/or number of the photosynthetic units, variations in the ratio of Chl *a* to other pigments, and changes in cell morphology (Platt and Jassby 1976; Perry et al. 1981; Falkowski 1981). Increases in the cellular content or relative proportion of Chl *a* as cells adapt to low light would tend to decrease the Chl *a* normalized initial slope, α. Consequently, a lower α represents a more efficient light gathering capability.

Although we did not find a correlation between α and light to suggest adaptation, it is possible that the changes in species composition throughout the year (Table 2) may have had the same result. For example, the mean cell volume and Chl *a* concentration per cell volume varied about twofold in our samples during the year. Although there was no correlation of α with Chl *a*/cell volume, the correlation with mean cell volume was significant with a negative slope (-0.876, $p < 0.01$, $n = 8$). Our finding of an inverse relationship between α and mean cell volume was probably aided by the year-round dominance of pennate diatoms in our samples. Thus, changes in cell shape with species, which may also affect the magnitude of α, were not a complication.

Inhibition

Photosynthetic inhibition can occur when cells are exposed to light levels above those required for saturation. The effects include increases in rates of respiration and excretion together with photooxidation and bleaching of chlorophyll, with the severity of the effects dependent on the time of exposure (Harris 1980). Inhibition has frequently been observed for phytoplankton, in both in situ and incubator experiments. There has been concern, however, whether the methodology used neglects the mixing that phytoplankton may experience within the euphotic zone. Thus, the time during which cells are subjected to high light may be artificially increased by the incubation procedures (Marra 1978). For periphyton, this particular problem does not normally occur, as cells are usually fixed with respect to the incident light. Thus, results from fixed in situ or incubator experiments would not suffer from the same inherent qualifications. There is another potential problem, however, whenever periphyton suspensions are used because dense suspensions can attenuate the actual degree of inhibition. This has been generally overlooked in work with epipelic suspensions, and details of actual cell densities have frequently been omitted. In our experiments, therefore, we maintained a low and relatively constant concentration of cells (as measured by Chl *a*) so that self-shading effects were minimized and all the results were comparable. It should be noted here that these low concentrations would tend to favour the demonstration of photoinhibition, and thus extrapolation of the results to periphyton mats has to be done with caution.

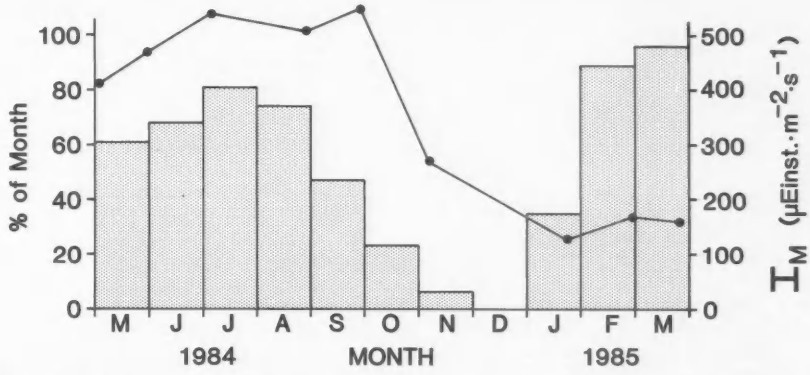


FIG. 5. Seasonal trend in the irradiance optimal for photosynthesis, I_m (●), together with the percent of each month that the incident irradiance was above I_m for more than half the daylight period.

Of the three parameters used to characterize the inhibition part of the $P(I)$ curve (Fig. 2), β is the least useful because it is directly affected by the magnitude of P^B_s, the potential maximum photosynthetic rate (Platt et al. 1980). It is included here because it is derived directly from the curve-fitting equation. A more useful representation of the degree of inhibition is the ratio P^B_{max}/β, represented by I_b, which varies inversely with the amount of inhibition. This parameter ranged from 1500 to 6900 μEinst.·m^{-2}·s^{-1} (Table 5). Using the conversion factor of 1 W·m^{-2} = 3.3 μEinst.·m^{-2}·s^{-1} (Colijn and van Buurt 1975), Platt et al. (1980) found a similar range of values, 1200−14 000 μEinst.·m^{-2}·s^{-1}, for marine phytoplankton. Although comparable data for periphyton are unavailable, several workers have discovered decreased photosynthetic rates with high light in epipelic (Hunding 1971; Colijn and van Buurt 1975; King and Schramm 1976) and algae colonized on styrofoam (incubated in situ, Flint et al. 1977). In addition, Turner et al. (1983, his table 2) showed data indicating possible inhibition in epilithic samples.

Conversely, others have found no evidence of inhibition in periphyton (Eichenberger and Wuhrmann 1975; Hornberger et al. 1976; Carpenter 1985). Hornberger et al. (1976), using a method based on free oxygen measurements, consistently found linear relationships between light and productivity in a small river periphyton community. Furthermore, they challenged the accuracy of bottle incubations, suggesting that nutrient limitation is responsible for reported light saturation and inhibition. This possibility may exist for relatively dense incubated samples, especially if the natural flow conditions are not established. However, for dilute suspensions, with adequate mixing, this is not a problem. Our time course experiment, for example, does not suggest any decrease in photosynthetic rates as a function of incubation time, which would be expected if the cells had become progressively nutrient starved.

There is a seasonal pattern in the degree of light inhibition, I_b, with highest values, representing minimum potential for inhibition, in the summer and lowest values in the winter (Table 5). The correlation with temperature is strong ($p < 0.01$, Table 6) but a weaker relationship is found with light. In the latter case there is only a significant correlation ($0.01 < p < 0.05$) between I_b and the 5- or 10-d average light data. The strong correlation with temperature is possibly due to the enhanced recovery from the effects of inhibition at increased temperature (Harris 1980). Also, inhibition is expected to be less severe in those cells that have a less efficient light gathering capability. (This is represented by higher levels of α because, as noted earlier, this Chl *a* normalized parameter varies inversely with efficiency.) Although α did vary throughout the year and, in 1984, was higher in the summer, we did not find a significant correlation with I_b.

A further representation of inhibition, I_m, corresponds to the light level optimal for photosynthesis and the level above which inhibition could occur. It is distinct from I_b in that it does not suggest the degree, if any, of potential inhibition. Seasonal trends in I_m were similar to I_b, with relatively high values from April through September (around 500) and low values from January through March (around 150 μEinst.·m^{-2}·s^{-1}, Table 5). Values during November seem to show a transition between these extremes.

Ecologically the magnitude of I_m is interesting because it appears to be substantially less than typical maximum ambient irradiance levels that would be expected throughout the year. For this reason the surface irradiance data (corrected for reflection losses, see Methods section) were examined for values that exceeded I_m. Days when half or more of the daylight period was above I_m were totalled for each month and expressed as a percentage of the total number of days during the month. It is apparent that there are two periods when the potential for in situ inhibition is strong, namely, the summer months and February−March (Fig. 5). It is clear from the high values of I_b (Table 5), however, that the periphyton populations during the summer show an adaptation to the higher ambient light levels. Thus, although UV light during the summer may be damaging to periphyton in shallow water (Bothwell, unpubl. data), the potential for inhibition by visible light is not realized at this time of year. Conversely, during late winter and early spring, low values of I_b would permit a relatively high degree of inhibition. At this time of year, then, light inhibition could have a serious impact on the rates of in situ periphyton photosynthesis.

In conclusion, we should stress that our results are based on lotic periphyton, grown under high light and moderately fast current conditions, with maximum densities less than 100 mg Chl *a*·m^{-2}. Caution is required in extrapolating these results to other habitats and especially to denser periphyton mats where diffusion characteristics and light availability may be quite different.

Nevertheless, we believe that the methodological approach used in this study offers significant improvements in the under-

standing of lotic periphyton photosynthesis. Thus, once the problems of sample heterogeneity and mixing during incubation have been reduced, it is clear that the photosynthetic characteristics of lotic periphyton are similar to other periphyton and indeed phytoplankton (especially those growing in high light near the water surface). Seasonal variations in Chl *a* normalized photosynthetic parameters, correlating with changes in temperature and irradiance, suggest that these lotic periphyton communities are, for the most part, tightly coupled to their environment. A notable exception to this occurs in late winter and early spring, during rapidly increasing irradiance levels, when there is a potential for serious light inhibition. Furthermore, we did not find any photosynthetic response to changes in the limiting nutrient, phosphorus. This is contrary to some of the previous studies of natural populations of phytoplankton.

Acknowledgements

We thank the Village of Chase, B.C., for permission to use their property for our field site. The technical assistance of Mary Bolin, Reg Mitchell, and Kelly Suzuki and the computer programming provided by Ron Wiegand are greatly appreciated. We also thank reviewer Michael Turner, who offered many constructive suggestions to improve this manuscript. This study was supported in part by a grant from Weyerhaeuser Canada Ltd. to M.L.B.

References

BEADALL, J., AND I. MORRIS. 1976. The concept of light intensity adaptation in marine phytoplankton: some experiments with *Phaedoactylum tricornutum*. Mar. Biol. 37: 377–387.

BOTHWELL, M. L. 1983. All-weather troughs for periphyton studies. Water Res. 17: 1735–1741.

1985. Phosphorus limitation of lotic periphyton growth rates: An intersite comparison using continuous-flow troughs (Thompson River system, British Columbia). Limnol. Oceanogr. 30: 527–542.

BOTHWELL, M. L., AND S. JASPER. 1983. A light and dark trough methodology for measuring rates of lotic periphyton settlement and net growth: an evaluation through intersite comparison, p. 253–265. *In* R. G. Wetzel [ed.] Periphyton of freshwater ecosystems. Dr. W. Junk Publishers, The Hague, The Netherlands.

BOTT, T. L., AND F. P. RITTER. 1981. Benthic algal productivity in a Piedmont stream measured by 14C and dissolved oxygen change procedures. J. Freshwater Ecol. 1: 267–277.

CARPENTER, R. C. 1985. Relationships between primary production and irradiance in coral reef algal communities. Limnol. Oceanogr. 30: 784–793.

COLIJN, F., AND G. VAN BUURT. 1975. Influence of light and temperature on the photosynthetic rate of marine benthic diatoms. Mar. Biol. 31: 209–214.

EICHENBERGER, E., AND K. WUHRMANN. 1975. Growth and photosynthesis during the formation of a benthic algal community. Verh. Int. Ver. Limnol. 19: 2035–2042.

FALKOWSKI, P. G. 1981. Light–shade adaptation and assimilation numbers. J. Plankton Res. 3: 203–216.

FEE, E. J. 1969. A numerical model for the estimation of photosynthetic production, integrated over depth and time, in natural waters. Limnol. Oceanogr. 14: 906–911.

FLINT, R. W., R. C. RICHARDS, AND C. R. GOLDMAN. 1977. Adaptation of styrofoam substrate to benthic algal productivity studies in Lake Tahoe, California–Nevada. J. Phycol. 13: 407–409.

GARGAS, E., C. S. NIELSEN, AND J. LØNHOLDT. 1976. An incubator method for estimating the actual daily planktonalgae primary production. Water Res. 10: 853–860.

GLOOSCHENKO, W. A., J. E. MOORE, M. MUNAWAR, AND R. A. VOLLENWEIDER. 1974. Primary production in Lakes Ontario and Erie: a comparative study. J. Fish. Res. Board Can. 31: 253–263.

HALLDAL, P. 1970. The photosynthetic apparatus of microalgae and its adaptation to environmental factors, p. 17–55. *In* P. Halldal [ed.] Photobiology of microorganisms. Wiley-Interscience, London.

HARDING, L. W., B. B. PRÉZELIN, B. M. SWEENEY, AND J. L. COX. 1982. Diel oscillations of the photosynthesis–irradiance ($P-I$) relationship in natural assemblages of phytoplankton. Mar. Biol. 67: 167–178.

HARRIS, G. P. 1980. The measurement of photosynthesis in natural populations of phytoplankton, p. 129–187. *In* I. Morris [ed.] The physiological ecology of phytoplankton. Blackwell Scientific Publications, Oxford, England.

HOLM-HANSEN, O., C. J. LORENZEN, R. W. HOLMES, AND J. D. STRICKLAND. 1965. Fluorometric determination of chlorophyll. J. Cons. Cons. Int. Explor. Mer 30: 3–15.

HORNBERGER, G. M., M. G. KELLY, AND R. M. ELLER. 1976. The relationship between light and photosynthetic rate in a river community and implications for water quality modeling. Water Resour. Res. 12: 723–730.

HORNER, R. R., E. B. WELCH, AND R. B. VEENSTRA. 1983. Development of nuisance periphytic algae in laboratory streams in relation to enrichment and velocity, p. 121–134. *In* R. G. Wetzel [ed.] Periphyton of freshwater ecosystems. Dr. W. Junk Publishers, The Hague, The Netherlands.

HUNDING, C. 1971. Production of benthic microalgae in the littoral zone of a eutrophic lake. Oikos 22: 389–397.

JASPER, S., M. L. BOTHWELL, AND R. MITCHELL. 1984. A multiple trough apparatus for year-round studies of periphyton. Inland Waters Directorate, Pacific and Yukon Region, Vancouver, B.C. 16 p.

JASPER, S., E. C. CARMACK, R. J. DALEY, C. B. J. GRAY, C. H. PHARO, AND R. C. WIEGAND. 1983. Primary productivity in a large, temperate lake with river interflow: Kootenay Lake, British Columbia. Can. J. Fish. Aquat. Sci. 40: 319–327.

JASSBY, A. D., AND T. PLATT. 1976. Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. Limnol. Oceanogr. 21: 540–547.

JONES, R. C., AND M. S. ADAMS. 1982. Seasonal variations in photosynthetic response of algae epiphytic on *Myriophyllum spicatum* L. Aquat. Bot. 13: 317–330.

KING, R. J., AND W. SCHRAMM. 1976. Photosynthetic rates of benthic marine algae in relation to light intensity and seasonal variations. Mar. Biol. 37: 215–222.

LOEB, S. L. 1981. An in situ method for measuring the primary productivity and standing crop of the epilithic periphyton community in lentic systems. Limnol. Oceanogr. 26: 394–399.

MARKER, A. F. H. 1976. The benthic algae of some streams in southern England. II. The primary production of the epilithon in a small chalk-stream. J. Ecol. 64: 359–373.

MARRA, J. 1978. Phytoplankton photosynthetic response to vertical movement in a mixed layer. Mar. Biol. 46: 203–208.

MCINTIRE, C. D. 1968. Structural characteristics of benthic algal communities in laboratory streams. Ecology 49: 520–537.

MCINTIRE, C. D., R. L. GARRISON, H. K. PHINNEY, AND C. E. WARREN. 1964. Primary production in laboratory streams. Limnol. Oceanogr. 9: 92–102.

MCINTIRE, C. D., AND H. K. PHINNEY. 1965. Laboratory studies of periphyton production and community metabolism in lotic environments. Ecol. Monogr. 35: 237–258.

MORRIS, I., C. M. YENTSCH, AND C. S. YENTSCH. 1971. Relationship between light carbon dioxide fixation and dark carbon dioxide fixation by marine algae. Limnol. Oceanogr. 16: 854–858.

PERRY, M. J., M. C. TALBOT, AND R. S. ALBERTE. 1981. Photoadaption in marine phytoplankton: response of the photosynthetic unit. Mar. Biol. 62: 91–101.

PFEIFER, R. F., AND W. F. MCDIFFETT. 1975. Some factors affecting primary productivity of stream riffle communities. Arch. Hydrobiol. 75: 306–317.

PLATT, T., K. L. DENMAN, AND A. D. JASSBY. 1977. Modeling the productivity of phytoplankton. *In* E. D. Goldberg [ed.] The sea: ideas and observations on progress in the study of the sea. Wiley, New York, NY.

PLATT, T., C. L. GALLEGOS, AND W. G. HARRISON. 1980. Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. J. Mar. Res. 38: 687–701.

PLATT, T., AND A. D. JASSBY. 1976. The relationship between photosynthesis and light for natural assemblages of coastal marine phytoplankton. J. Phycol. 12: 421–430.

PLATT, T., AND D. V. SUBBA RAO. 1975. Primary production of marine microphytes, p. 249–280. *In* J. P. Cooper [ed.] Photosynthesis and productivity in different environments. Cambridge University Press, Cambridge.

REDALJE, D. G., AND E. A. LAWS. 1981. A new method for estimating phytoplankton growth rates and carbon biomass. Mar. Biol. 62: 73–79.

ROBINSON, G. G. C. 1983. Methodology: the key to understanding periphyton, p. 245–251. *In* R. G. Wetzel [ed.] Periphyton of freshwater ecosystems. Dr. W. Junk Publishers, The Hague, The Netherlands.

RODGERS, J. H. JR., K. L. DICKSON, AND J. CAIRNS, JR. 1978. A chamber for *in situ* evaluations of periphyton productivity in lotic systems. Arch. Hydrobiol. 84: 389–398.

SCHINDLER, D. W., V. E. FROST, AND R. V. SCHMIDT. 1973. Production of epilithiphyton in two lakes of the Experimental Lakes Area, northwestern Ontario. J. Fish. Res. Board Can. 30: 1511–1524.

STAINTON, M. D. 1973. A syringe gas-stripping procedure for gas-chromatograph determination of dissolved inorganic and organic carbon in fresh water and carbonates in sediments. J. Fish. Res. Board Can. 30: 1441–1445.

STEELE, J. H. 1962. Environmental control of photosynthesis in the sea. Limnol. Oceanogr. 7: 137–150.

TALLING, J. F. 1957. The phytoplankton population as a compound photosynthetic system. New Phytol. 56: 133–149.

THOMAS, N. A., AND R. L. O'CONNELL. 1966. A method for measuring primary production by stream benthos. Limnol. Oceanogr. 11: 386–392.

THOMAS, W. H. 1969. Phytoplankton nutrient enrichment experiments off Baja California and in the eastern equatorial Pacific Ocean. J. Fish. Res. Board Can. 26: 1133–1145.

TURNER, M. A., D. W. SCHINDLER, AND R. W. GRAHAM. 1983. Photosynthesis–irradiance relationships of epilithic algae measured in the laboratory and *in situ*, p. 73–87. *In* R. G. Wetzel [ed.] Periphyton of freshwater ecosystems. Dr. W. Junk Publishers, The Hague, The Netherlands.

VOLLENWEIDER, R. A. 1965. Calculation models of photosynthesis–depth curves and some implications regarding day rate estimates in primary production measurement. *In* C. R. Goldman [ed.] Primary productivity in aquatic environments. University of California Press, Berkeley, CA.

WETZEL, R. G. 1964. A comparative study of the primary productivity of higher aquatic plants, periphyton and phytoplankton in a large, shallow lake. Int. Rev. Gesamten Hydrobiol. 49: 1–61.

1965. Techniques and problems of primary productivity measurements in higher aquatic plants and periphyton. Mem. Ist. Ital. Idrobiol. 18(Suppl.): 249–267.

WHITFORD, L. A., AND G. J. SCHUMACHER. 1961. Effect of current on mineral uptake and respiration by a fresh-water alga. Limnol. Oceanogr. 6: 423–425.

Winter Habitat Utilization by Stream Resident Brook Trout (*Salvelinus fontinalis*) and Brown Trout (*Salmo trutta*)

Richard A. Cunjak and Geoffrey Power

Department of Biology, University of Waterloo, Waterloo, Ont. N2L 3G1

Cunjak, R. A., and G. Power. 1986. Winter habitat utilization by stream resident brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*). Can. J. Fish. Aquat. Sci. 43: 1970–1981.

Habitat utilization by brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*) is described from three winters of underwater observations in a southern Ontario river. Older trout (>age 1) generally occupied positions in deeper and faster water than age 0+ trout. In winter, at sites of sympatry, brown trout occupied greater focal point water depths than brook trout; both species had similar focal point water velocities. At all sites, and for both age groups and species, there was a strong preference for positions beneath cover. Relative to summer, trout positions in winter were characterized by slower water velocities and greater overhead cover. In winter, most trout were in aggregations, usually in pools beneath cover and close to point sources of groundwater discharge. Gregarious behaviour appeared to increase as water temperatures decreased; no such relationship was evident in the summer. Specific strategies for overwintering varied between sites and age groups but generally conformed to the theory of energetic cost minimization for position choice. These variable patterns appear to be adaptive.

Les auteurs traitent de l'utilisation de l'habitat par l'omble de fontaine (*Salvelinus fontinalis*) et la truite brune (*Salmo trutta*) suite à trois hivers d'observations sous-marines effectuées dans un cours d'eau du sud de l'Ontario. Les truites plus âgées (âge 1+) se trouvaient généralement dans des eaux plus profondes et à écoulement plus rapide que celles d'âge 0+. En hiver et dans les lieux de sympatrie, les truites brunes occupaient des profondeurs plus focalisées que les ombles de fontaine; la focalisation par rapport aux vitesses d'écoulement était semblable chez les deux espèces. Les deux groupes d'âge des deux espèces présentaient une forte préférence pour les lieux sous couvert, ceci à tous les sites. Les poissons préféraient des vitesses d'écoulement moins importantes et un couvert plus important en hiver, comparativement à l'été. En hiver, la plupart des poissons se regroupaient, généralement dans des fosses sous couvert, à proximité des sources ponctuelles d'eau souterraine. Ce comportement grégaire semblait s'accroître à mesure que la température diminuait; une relation de ce type n'a pas été notée en été. Les stratégies adoptées au cours de l'hiver variaient selon les sites et les groupes d'âge, mais elles se conformaient généralement à la théorie de la minimisation des coûts énergétiques lors du choix d'un emplacement. Il semble que ces comportements variables soient de nature adaptative.

Received March 17, 1986
Accepted July 3, 1986
(J8717)

Reçu le 17 mars 1986
Accepté le 3 juillet 1986

Direct underwater observations, for use in freshwater fisheries biology, have been performed since the early 1960's (Keenleyside 1962; Northcote and Wilkie 1963). The usefulness of this technique for observing species in their natural environment is responsible for its increasing popularity in recent years over a range of locations and situations (Griffith 1981; Cunjak and Green 1983; Moyle and Baltz 1985). Winter studies, however, have been the exception. Despite the early realization of the importance of and the lack of information for this season (Hubbs and Trautman 1935), the number of investigations is few and these have been limited to lakes (Emery 1978), West Coast rivers with little or no ice formation (Bustard and Narver 1975a; Tschaplinski and Hartman 1983), or deductions about winter behaviour based on autumnal observations (Rimmer et al. 1983, 1984). In temperate latitude streams subject to freezing conditions for considerable periods (months) over the winter, there exists no published record of salmonid behaviour based on underwater observations.

Knowledge of a species' winter habitat is requisite to fisheries management and predictive modelling (Bovee 1978, 1982; Binns and Eiserman 1979). Lack of data for any part of the year precludes the realization of these models' full potential. The importance of such data is emphasized if, as Chapman (1966) asserted, space (i.e. habitat) is the prime regulator of stream salmonid populations in winter. To the fisheries manager, therefore, information on preferred habitats in all seasons increases the probability for success in stocking programs and stream enhancement schemes.

For this study, "winter" has been defined as that period extending from the time immediately following the autumn spawning of brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*) (coincident with a decline in water temperatures) and extending until a steady increase in water temperatures in spring exceeds a diel mean of 4.0°C (usually accompanied by a major spate). Such a definition has more biological meaning than one following calendar dates. Over the three winters encompassing our research, "winter" extended from the last week in November until the last week in March.

Diving observations were undertaken at three sites in the headwaters of the Credit River. The primary objective was to describe the selected winter habitat and behaviour of brook trout and brown trout. Two age groups, 0+ and older trout (i.e. >1), were considered separately for each species and the re-

FIG. 1. Location of study sites in the headwaters of the Credit River drainage. 1, Caledon; 2, Spring Tributary; 3, North Branch.

spective habitat preferences compared. For the purposes of this project, fish that had not yet completed their first winter were designated 0+ even though they were past the nominal January 1 birthdate (Jearld 1983). We tested the hypothesis that winter habitat utilization differed between age groups within a species. Similar studies on blacknose dace (*Rhinichthys atratulus*) (Cunjak and Power 1986a), Pacific salmonids (*Oncorhynchus kisutch* and *Salmo gairdneri*) (Bustard and Narver 1975a), and Atlantic salmon (*Salmo salar*) (Rimmer et al. 1983, 1984) suggest that winter behaviour can differ between stream species, or even within a species over a latitudinal gradient (Cunjak and Power 1986b). Therefore, a second hypothesis, that winter habitat differed for brook and brown trout, was also tested by comparing observations at the two sites of sympatry.

Study Area and Site Descriptions

The Credit River, a fifth-order stream in southern Ontario, drains an area of 850 km^2 and discharges into Lake Ontario at 43°33′N, 79°33′W. The physiography throughout much of the headwater region including the study area is characterized by a network of glacial spillways containing deposits of gravel, often overlain with sand and silt (Portt 1982). Mean annual precipitation and snowfall varies from 71 to 86 mm and 122 to 203 mm, respectively; mean daily air temperatures are normally between −3 and −8°C in January and between 26 and 27°C in July (Portt 1982). The riparian community at the study sites is dominated by white cedar; mixed hardwoods (beech, maple, poplar) and hemlock are also present but much less common.

Brook trout is the only extant native salmonid in the Credit River. In the vicinity of the Forks of the Credit (Fig. 1), brook trout, rainbow trout, and brown trout have been stocked regularly since before 1938 to as recently as 1979 (Portt 1982). Above the Niagara Escarpment (a barrier to fish migration) on Black Creek and the North and West branches of the main river, only brook trout were stocked but not since 1971.

Three sites (Fig. 1) were selected because they provided variable species assemblages and habitat characteristics. Herein, these sites are referred to as Caledon, Spring Tributary, and the North Branch.

Caledon (Fig. 1, No. 1) is situated 2.5 km above the edge of the Niagara Escarpment. Brook trout is the only salmonid species present. Other common fish species include white sucker (*Catostomus commersoni*), blacknose dace, and longnose dace (*Rhinichthys cataractae*). The stream gradient is the lowest of all sites (3.10 m·km^{-1}) and the substrate is mainly sand, gravel, and rubble. Mean summer and winter discharges (1982–85) were 1.63 and 2.24 m^3·s^{-1}, respectively (Environment Canada 1985). Maximum (August) and minimum

(February–March) recorded water temperatures were 22.0 and 0.1°C, respectively. Surface ice first formed along stream edges in late November and usually persisted until March. Anchor ice was observed each winter in exposed riffles and runs but was not common. Mean surface ice cover (i.e. the percentage of wetted surface covered by ice) was 24% and covered the shallow stream margins (<20 cm deep) and back-eddy pools where surface flow was negligible; rarely did ice extend across the width of the stream. Monthly water analyses in 1984 indicate that this site is slightly alkaline (pH range 7.7–8.3). Turbidity was generally low except during spates such as in the late winter when a value of 50 N.T.U. was recorded. The annual range of conductivity and total hardness was 0.45–0.75 (mS) and 160–260 mg $CaCO_3 \cdot L^{-1}$, respectively.

Spring Tributary (Fig. 1, No. 2) is a second-order tributary of the North (main) Branch located 2.0 km upstream of the Forks of the Credit. Brook trout are the predominant fish species but brown trout, blacknose dace, and white sucker are also present. Snorkeling was confined to the lower 200 m of stream where the substrate is mainly sand with dense mats of watercress (*Nasturtium officinale*) along the stream margins. A high contribution to the streamflow from proximal groundwater sources (Cunjak, unpubl. data) is responsible for minimal annual fluctuation in water levels and temperatures. Mean discharge is low (0.0679 $m^3 \cdot s^{-1}$). Ice formation was never observed, as the minimum recorded water temperature was only 1.5°C (February, March). Maximum summer temperature was 16.5°C (July), turbidity is very low (1–6 N.T.U.), pH ranges between 7.7 and 8.1, conductivity is 0.45–0.60 mS, and total hardness varies from 165 to 260 mg $CaCO_3 \cdot L^{-1}$.

North Branch (Fig. 1, No. 3) is that section of the Credit River between Alton and the junction with the West Credit River; the site of snorkeling was 2.0 km upstream from the Forks of the Credit and only 45 m downstream from the mouth of the Spring Tributary. Fish species diversity is the highest of all sites. The common species include brook trout, brown trout, blacknose and longnose dace, white sucker, and mottled sculpin (*Cottus bairdi*). Stream gradient is relatively high (16.0 $m \cdot km^{-1}$) and substrate is mainly boulder and rubble. The discharge is similar to that given for the Caledon site, only 3.5 km upstream. Water temperatures reach a maximum of 22.0°C (July–August) and a minimum of 0.1°C (December–March). Anchor ice formation occurred more frequently here than at the Caledon site. Mean surface ice cover was 22% and ice occasionally bridged the stream. Maximum turbidity was measured here (86 N.T.U.) in late winter. Values of other parameters were pH 7.9–8.4, conductivity 0.45–0.60 mS, and total hardness 200–260 mg $CaCO_3 \cdot L^{-1}$.

During the winters of 1983–84 and 1984–85, water temperatures were monitored on continuous recording Peabody-Ryan thermographs at the Spring Tributary and North Branch sites. Water temperatures were also taken with pocket thermometers during all field trips. Dissolved oxygen was at or near saturation at all sites throughout the course of this study.

Methods

Underwater observations were made using a dry suit, mask, and snorkel over three winters with quantitative observations in 1983–84 and 1984–85. Dives were made from December 8 to March 29 between 10:00 and 14:30. At least 2 d separated consecutive observations to reduce any disturbance effect caused by the diver. Observations lasted 20–60 min depending on the number of fish seen and the length of the study area. A minimum underwater visibility of 1.5 m was required for a dive. This value was the minimum distance that a fish could be approached before the fish's behaviour was affected by the diver. Each dive was initiated from the downstream end of the study area. The diver then moved slowly in an upstream direction along predetermined zig-zag transects incorporating all available habitat types (riffles, runs, pools, undercuts, etc.).

When a fish was encountered, it was observed for up to 5 min during which the following were noted: species, size, age group (0+ or older). Fish size was determined by associating snout and tail positions with adjacent objects and then measuring the distance. With practice, visual estimates of size could be made without direct measurements. Identification of age groups was based on relative size differences and external body patterns (parr marks). General behavioural observations of agonism, feeding, movement, and position (resting on the substrate or hovering over station) were also noted. Despite the cautious approach of the diver along transects, some fish were frightened from their stations. The positions of such visibly disturbed fish were disregarded.

The location of each fish's focal point (Fausch and White 1981) was marked with a coloured stone. Following an observation period, focal point water velocity and water depth (from water surface to focal point) were measured at the approximate head position of the fish. Since focal points were almost always close to the stream bed (i.e. within 15 cm), they were also an indicator of chosen water depths. A portable flowmeter (described by Edington and Molyneux 1960) was used to measure water velocity.

Association to cover was assessed for all station-holding fish. In this study, cover was defined as (1) a "broken" water surface caused by turbulence or high water velocity, (2) logs, algae, macrophytes, boulders, or any submerged structure beneath which a fish could be hidden from a view overhead, (3) undercut banks or a riparian canopy <1 m above the water surface, or (4) surface ice. A fish was considered under cover if directly beneath any of these or near enough that it provided shade.

During the first winter's observations, some trout were seen in groups or "aggregations" behaving as cohesive units rather than as individuals. Since this was relatively common for both brook and brown trout, it was decided to treat groups as discrete entities, and so habitat utilization was described separately for individuals and aggregations. An aggregation is herein defined as "a group of fish of one or more species, in close association (within approximately one body length of each other), displaying a common behavioural pattern but lacking the spatial homogeneity and polarity of schooling fish." This is similar to the "non-polarized school" described by Keenleyside (1979). The number of fish in an aggregation was variable but five fish were considered the minimum number. Gregarious behaviour was not apparent for smaller groups in a localized area. Instead they occupied more defined territories, or at least separate stations.

Focal point depth and water velocity for aggregations were determined by taking the mean of measurements at the anterior, posterior, and centre of the group. Cover association was based on the position of the majority of the fish within an aggregation.

TABLE 1. Habitat characteristics at each of three study sites on the Credit River during winter. B = boulder; R = rubble; G = gravel; S = sand; Si = Silt; L = logs/branches; M = macrophytes/algae.

Study site	Length (m)	Mean width ± SE (range) (m)	Mean depth ± SE (range) (cm)	Mean water velocity ± SE (range) ($cm \cdot ^{-1}$)	Mean % cover	Substrate type (%)						
						B	R	G	S	Si	L	M
Caledon	88.0	12.3±0.44 (9.8−14.8)	68.4±5.46 (19.0−150.0)	22.2±2.72 (1.5−60.4)	25.0	5	20	23	43	4	5	0
Spring Tributary	56.0	5.2±0.41 (3.6−8.1)	25.6±1.76 (11.0−46.0)	11.6±2.30 (1.5−63.4)	42.5	3	8	10	29	14	2	34
North Branch	60.0	9.4±0.60 (7.0−12.7)	48.2±3.42 (15.0−90.0)	42.5±3.59 (1.5−94.1)	61.0	43	28	14	12	1	0	1

TABLE 2. Frequency and mean size (total length, TL) of brook trout and brown trout observed during snorkeling at each site in winter. Only the ranges of sizes are given for aggregations.

Species	Age classification	n	Mean size (cm TL) ± SE (range)		
			Caledon	Spring Tributary	North Branch
Brook trout	0+	62	8.3±0.49 (6.0−11.0)	8.7±0.20 (6.0−12.0)	8.6±0.51 (7.0−10.0)
	>1	82	19.6±0.98 (11.0−30.0)	15.2±0.47 (12.0−22.0)	17.5±0.89 (15.0−20.0)
Brown Trout	0+	20	—	11.6±0.24 (10.0−12.0)	11.1±0.39 (9.0−12.0)
	>1	52	—	20.3±2.52 (12.0−40.0)	21.9±1.58 (12.0−50.0)
Aggregations[a]		1411	(6.0−30.0)	(7.0−25.0)	(7.0−30.0)

[a]Includes both trout species and life stages.

To compare habitat utilization between seasons, snorkeling observations were also performed during the summer of 1984. These dives were made from June 13 to August 16 between 10:00 and 15:00. All procedures were similar to those used in winter.

Descriptions of stream habitat at each of the study sites (Table 1) were based on 30 transects according to the format proposed by Orth (1983).

Statistics

Hypotheses about habitat use by life stages, species, or seasons were tested using Student's t-test (depth flow) or by G-tests of independence for 2 × 2 contingency tables (cover) as described by Zar (1974). The testing of intersite differences in observed fish size was by t-test (brown trout) and ANOVA (brook trout). Interseasonal comparisons of fish abundance and feeding/resting frequency were analysed by t-tests and 2 × 2 G-tests, respectively. Linear regression was used for assessing the relationship of fish size and depth or flow as well as between water temperature and aggregation size. Assumptions of normality and homoscedasticity were checked for all parametric tests. Where such assumptions were violated, an approximate t-test for unequal variances (SAS Institute Inc. 1982) or a nonparametric Kruskal−Wallis test was used.

Results

A total of 55 dives was made during winter among the three sites (Spring Tributary = 23; Caledon and North Branch = 16 each) accounting for over 45 h of underwater observations. Habitat quantification was carried out for 144 individual (station-holding) brook trout and 72 individual brown trout. A further 1355 trout (species combined) were found in winter aggregations (Spring Tributary = 716; North Branch = 503; Caledon = 136). Most of the salmonids observed at the sites during winter were, therefore, in aggregations; individual trout (species combined) made up only 26, 13 and 10% of all fish quantified at the Caledon, Spring Tributary, and the North Branch sites, respectively. Water temperatures during winter snorkeling observations ranged between 1.5 and 5.5°C (Spring Tributary) and between 0.1 and 3.5°C (North Branch and Caledon).

Since trout growth is negligible in streams over the winter (Cooper 1953; Mortensen 1977), it was assumed that sizes were comparable throughout this season. Estimated sizes of 0+ brook trout were similar among the three sites (Table 2). Brown trout fry tended to be larger, reflecting their greater growth rates (see review in Carlander 1969). Older brook trout showed a range of mean sizes from 15.2 to 19.6 cm total length (TL), being largest at the Caledon site; older brown trout were larger. Aggregations, which included fry and overyearling trout of both species, showed a wide range of sizes (Table 2) although large brown trout (i.e. >30 cm TL) were not observed in such groups.

In winter, 0+ brook trout occupied shallower water ($p < 0.001$) than did older fish at two of the sites (Table 3). At the Spring Tributary, the mean focal point depth of 0+ trout was

TABLE 3. Winter habitat utilization between age classifications of brook trout and brown trout at three sites on the Credit River. Parenthetical values are ranges of the variable. $*p < 0.05$; $**p < 0.01$; ns = not significant.

Study site	Age classification	n	Mean focal point depth (cm)		Mean focal point velocity ($cm \cdot s^{-1}$)		Association beneath cover (%)	
			Brook trout					
Caledon	0+	11	31.2±3.15 (25.0−60.0)	**	4.3±0.89 (1.5−10.5)	*	100.0	ns
	>1	37	95.4±3.84 (30.0−150.0)		7.3±0.82 (1.5−22.5)		91.4	
Spring Tributary	0+	44	40.3±1.52 (20.0−67.0)	ns	3.0−0.22 (1.5−6.0)	**	79.6	ns
	>1	39	42.4±1.32 (25.0−60.0)		4.6±0.44 (1.5−18.0)		84.6	
North Branch	0+	7	27.6±4.3 (20.0−53.0)	**	1.5±0.00 (1.5−1.5)	**	100.0	ns
	>1	6	71.5±7.68 (34.0−81.0)		17.8±3.43 (4.0−23.0)		83.3	
			Brown trout					
Spring Tributary	0+	11	46.2±3.34 (30.0−63.0)	ns	4.7±0.69 (1.5−9.5)	ns	100.0	ns
	>1	16	53.4±3.20 (29.0−68.0)		5.7±1.24 (1.5−18.0)		100.0	
North Branch	0+	9	43.1±2.97 (26.0−55.0)	**	2.2±0.44 (1.5−4.5)	**	100.0	ns
	>1	36	75.6±5.96 (31.0−150.0)		16.0±1.70 (1.5−43.0)		88.89	

TABLE 4. Regression relationships of trout total length (X, cm) versus the habitat variables (Y) of water depth and water velocity measured during winter at each site.

Study site	Dependent variable	Equation	p	Total df	r^2
		Brook trout			
Caledon	Depth	$Y = 3.727X + 18.114$	<0.001	43	0.616
	Velocity	$Y = 0.083X + 5.299$	0.410	43	0.016
Spring Tributary	Depth	$Y = 0.212X + 38.397$	0.421	79	0.008
	Velocity	$Y = 0.169X + 1.681$	0.012	79	0.078
North Branch	Depth	$Y = 3.684X + 3.349$	0.019	10	0.477
	Velocity	$Y = 1.601X - 11.170$	0.006	10	0.585
		Brown trout			
Spring Tributary	Depth	$Y = 0.394X + 43.844$	0.159	26	0.078
	Velocity	$Y = 0.047X + 4.491$	0.612	26	0.010
North Branch	Depth	$Y = 0.070X + 67.540$	0.904	42	0.001
	Velocity	$Y = 0.742X + 1.429$	<0.001	42	0.452

also less but the difference was not significant ($p = 0.302$). At all three sites, older brook trout occupied winter habitats with higher flows than were measured for 0+ ($p < 0.05$, Table 3). This apparent positive relationship between fish size and focal point depths or flows during winter was tested by linear regression (Table 4). Four of the six regressions for brook trout were significant ($p < 0.02$). The strongest relationships (Caledon, depth; North Branch, flow) were found at those sites where the respective habitat variables had the greatest ranges among all sites (Table 1). However, it should be noted that the coefficient of determination (r^2) was rather low in all cases with no more than 62% of the total variation in any regression being attributable to the fit of the line. The percentage of individual brook trout beneath cover in winter was high (at least 79%) with no significant differences ($p > 0.75$) between age groups at any of the sites (Table 3).

Like brook trout, older brown trout were characterized by deeper and faster water velocity focal points than were 0+ (Table 3), but the differences were only significant ($p < 0.01$) at the North Branch site. Regressions of brown trout size versus flow (Table 4) yielded a positive relationship ($p < 0.001$) at the North Branch. No significant relationship ($p > 0.15$) was

TABLE 5. Seasonal comparisons of habitat utilization by brook trout and brown trout at different sites on the Credit River. Statistical significance values are for between-season comparisons. S = summer; W = winter; $*p < 0.005$; $**p < 0.01$; ns = not significant.

Study site	Age classification	Season	*n*	Mean focal point depth (cm)	Mean focal point velocity ($cm \cdot s^{-1}$)	Association beneath cover (%)
			Brook trout			
Caledon	0+	S	69	67.2 **	5.8 ns	34.8 **
		W	11	31.2	4.3	100.0
	>1	S	79	91.9 ns	8.9 *	67.1 **
		W	37	95.4	7.3	91.4
Spring Tributary	0+	S	107	34.9 **	4.7 **	70.1 ns
		W	44	40.3	3.0	79.6
	>1	S	34	36.7 *	4.7 ns	97.1 ns
		W	39	42.4	4.6	84.6
North Branch	0+	S	15	31.7 ns	2.9 ns	100.0 ns
		W	7	27.6	1.5	100.0
	>1	S	32	59.5 ns	15.1 ns	65.6 ns
		W	6	71.5	17.8	83.3
			Brown trout			
Spring Tributary	0+	S	0	—	—	—
		W	11	46.2	4.7	100.0
	>1	S	5	43.0 ns	5.4 ns	100.0 ns
		W	16	53.4	5.7	100.0
North Branch	0+	S	54	42.0 ns	13.6 **	72.2 *
		W	9	43.1	2.2	100.0
	>1	S	22	58.6 *	17.2 ns	90.9 ns
		W	36	75.6	16.0	88.9

found between brown trout size and focal point depth at this site or at the Spring Tributary for either depth or flow. Both 0+ and older brown trout frequently held positions beneath cover (Table 3), especially in relation to the availability of the resource at each site (Table 1).

At the two sites where they were sympatric, brown trout occupied greater focal point water depths than brook trout, but the difference was only significant ($p < 0.01$) for 0+ fish at the North Branch and older fish at the Spring Tributary. Focal point water velocities over the winter were similar, as the only significant difference ($p < 0.01$) was measured at the Spring Tributary where 0+ brown trout occupied positions in the faster flows. Although not significant, 0+ brown trout at the North Branch also occupied a higher mean focal point velocity. This trend was less obvious among older brown trout which held stations in the fastest water (up to 43.0 $cm \cdot s^{-1}$ compared with a maximum of 23.0 $cm \cdot s^{-1}$ for brook trout, Table 3). There were no significant ($p > 0.10$) interspecific differences for cover association for either life stage at either site; both species showed a high use of cover in winter.

Eight dives were made at each site during the summer of 1984. Water temperatures ranged between 17.0 and 22.0°C (Caledon), 15.0 and 22.0°C (North Branch), and 11.0 and 16.0°C (Spring Tributary). Both species were more abundant (or at least more were seen) in summer than winter (Fig. 2), largely due to the seasonal abundance of 0+ fish (brook trout at Caledon and the Spring Tributary; brown trout at the North Branch). The lowest mean number of trout seen per dive was at the Caledon site in both summer and winter (Fig. 2). The

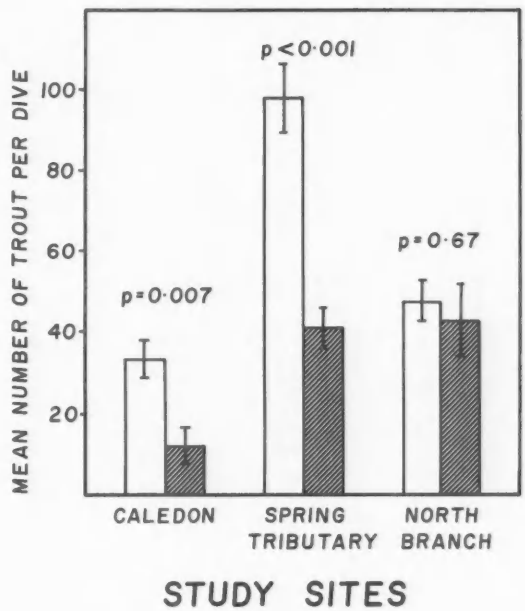


FIG. 2. Mean number of trout (species combined) observed per dive in the summer (open bars) and winter (shaded bars) at each site. Vertical bars represent the standard error. Probability values are for interseasonal comparisons of abundance at each site.

TABLE 6. Seasonal comparisons of group size and habitat utilization by aggregated trout (*Salvelinus fontinalis* and *Salmo trutta*) at sites on the Credit River. Statistical significance values are for between-season comparisons. S = summer; W = winter; parenthetical values are the ranges of particular variables. $*p < 0.05$; ns = not significant.

Study site	Season	Number of aggregations observed	Ratio number of brook trout/ brown trout	Mean size of aggregation (no. of trout) ± SE (range)	Mean focal point depth ± SE (range) (cm)	Mean focal point velocity ± SE (range) ($cm \cdot s^{-1}$)	% beneath cover ± SE
Caledon	S	13	108/0	8.1±0.87 (5–15) *	91.2±6.34 (40–115) ns	11.1±1.66 (1.5–21.0) *	42.9 *
	W	7	136/0	19.4±4.48 (5–40)	75.0±17.50	6.1±0.95 (3.8–11.0)	100.0
Spring Tributary	S	39	601/1	15.8±1.79 (5–50)	43.8±1.63 (22–60)	6.6±0.68 (1.5–19.0) *	50.0 *
	W	45	686/30	15.8±1.45 ns (5–46)	47.4±1.51 ns (20–65)	4.2±0.62 (1.5–25.0)	75.6
North Branch	S	10	229/28	25.7±3.94 (5–39)	54.4±2.37 (48–73)	7.7±0.58 (5.0–11.0) ns	100.0 ns
	W	19	427/76	29.3±3.57 ns (10–55)	49.7±2.75 ns (26–72)	6.1±1.14 (1.5–17.0)	94.7

especially low numbers in winter appeared at least partly due to emigration to a series of slow, deep (>2 m) steadies (flats) 0.4 km downstream where subsequent underwater observations indicated that many overyearling brook trout and white sucker were present throughout the winter.

Focal point water depths utilized by 0+ brook and brown trout showed no consistent trends between seasons (Table 5). At the Caledon site, 0+ trout were in shallower ($p < 0.001$) water during the winter although it should be noted that 9 of these 11 fish were found in a very small, shallow side-braid to the main channel, an atypical portion of the study site, rarely utilized by 0+ or older trout during the summer. At the Spring Tributary, 0+ brook trout were in deeper water ($p < 0.01$) in the winter whereas North Branch 0+ fish of both species showed no significant interseasonal depth differences ($p > 0.50$). Older brook and brown trout held stations in deeper water over the winter. This situation was the same at all sites although it was only significant ($p < 0.05$) at the Spring Tributary (brook trout) and at the North Branch (brown trout).

Water velocities at focal points were generally less in winter than summer for both species and life stages (Table 5). Significant differences ($p < 0.05$) occurred at Caledon (older trout), the Spring Tributary (0+ brook trout), and at the North Branch (0+ brown trout). The highest focal point velocities were measured at the North Branch site in each season and for both species.

Association to cover at each site was often less in the summer than in winter (Table 5). This was most obvious at the Caledon site where both 0+ and older brook trout showed significant differences ($p < 0.01$). At the North Branch, 0+ brown trout displayed a higher ($p < 0.05$) cover association in winter.

Both species were involved in aggregations although brook trout predominated in each instance of mixed aggregations. Brown trout made up less than 5% of trout in aggregations in summer and less than 15% in winter. Both 0+ and older trout were observed in aggregations, either in mixed assemblages or as separate groups. The particularly abundant 0+ brook trout populations found at the Spring Tributary in the summer accounted for a large proportion of the aggregations seen then and also contributed to the high numbers of trout enumerated during the dives (Fig. 2). The percentage of trout observed in summer aggregations was 42, 80, and 68% at Caledon, Spring Tributary, and North Branch sites, respectively. Trout displayed a greater propensity to aggregate in winter. Aggregations were larger at both the Caledon and North Branch sites although only significantly so ($p < 0.05$) at the former (Table 6).

Since the apparent abundance (density) of fish was less in the winter at all sites (Fig. 2), it was hypothesized that some factor(s), namely a lowering of water temperatures, might be influencing gregarious behaviour. The effect of temperature on size of aggregations in each season was tested by linear regression. Data from all sites were pooled for the analyses. In winter, a significant ($p < 0.001$) negative relationship was found (Fig. 3). As temperatures declined from 5.5 to 0.1°C during winter, the size of aggregations increased although only 24% of the observed variation could be explained by the fit of the line. In summer, no significant regression ($p = 0.837$) was found over the water temperature range of 11.0–22.0°C. The importance of water temperature as one of the factors influencing gregarious behaviour in winter is further supported by circumstances at the North Branch site. Of the 19 aggregations, 18 were found facing into the mouths of first-order spring tributaries or groundwater seeps discharging into the main stream. At these sites, localized water temperatures (at the aggregation focal point) were between 2.0 and 6.0°C warmer than in the main stream proper, usually only a metre from the aggregated fish.

Mean focal point depths and velocities measured for winter aggregations were in water deeper and slower than the respective site means for these variables (see Tables 1 and 6). This reflects the tendency for aggregations to occur in pools or runs, never in riffles. Aggregations were characteristically in slower flows with more cover in winter than in summer (Table 6). No significant difference ($p > 0.05$) was found for focal point depths between summer and winter, at any of the sites.

It was hypothesized that since water temperatures are colder in winter, feeding would be less and this would influence positions occupied by trout. This was tested by comparing the numbers of trout seen feeding during the two seasons. Position choice was tested by comparing the number of fish resting on

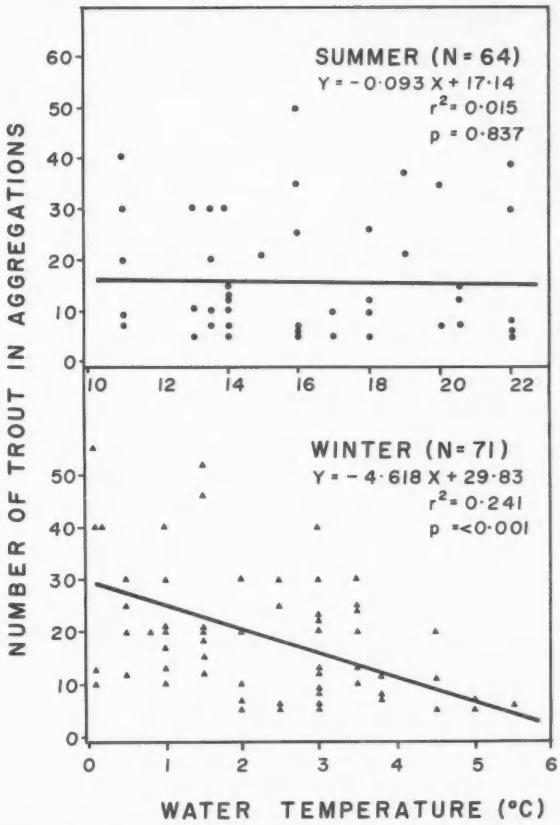

SUMMER (N = 64)
Y = -0·093 X + 17·14
r² = 0·015
p = 0·837
WINTER (N = 71)
Y = -4·618 X + 29·83
r² = 0·241
p = <0·001
NUMBER OF TROUT IN AGGREGATIONS
WATER TEMPERATURE (°C)

FIG. 3. Regression plots of the number of trout in an aggregation at different water temperatures during summer and winter. Data for all sites and species were pooled for the analyses. N = the number of aggregations observed in each season.

the substrate (assumed a nonfeeding, resting position) with those that hovered or held stations above the substrate. Data from all sites were pooled for the analyses. As hypothesized, the number of trout seen feeding in winter was less ($p < 0.001$) than in summer (Fig. 4). This probably accounts for the higher number of trout (both species) observed resting on the substrate ($\rho < 0.001$). Trout in aggregations were not included in the position choice analysis, as their behaviour was too variable (i.e. in any one aggregation some trout were resting on the stream bottom while others were not and the proportions changed continually during observations).

No behavioural or habitat utilization differences were noted over the winter; patterns identified in December were the same as those observed in March. Agonistic bouts were only seen at the Spring Tributary and only on two occasions. In both cases, the bouts were between brook trout in an aggregation and involved infrequent lateral displays and chases.

Other species found during winter included blacknose and longnose dace, mottled sculpin, juvenile white sucker, and leopard frogs (*Rana pipiens*). At the Caledon and North Branch sites, these were overwintering beneath rubble and boulders when water temperatures declined below 5.0°C in the autumn. All were in a semi-torpid state and were easily captured by hand. Larger white sucker were found in aggregations in the deepest pools.

Discussion

Habitat utilization by brook trout in winter differed between sites and age groups but generally conformed to the theory of energetic cost minimization for position choice (Smith and Li 1983; Bachman 1984; Fausch 1984) formulated from summer studies of stream salmonids. Both species of trout were observed in pools, the slow reaches of runs, or along stream margins. Rarely were trout seen holding positions in midstream, riffles, or the heads of pools, which are more typical of summer feeding stations. Tschaplinski and Hartman (1983) found that juvenile coho salmon did not occupy midstream locations during winter, preferring, instead, areas of low water velocity and cover. Mean focal point water velocities of Credit River trout in winter were consistently lower than the respective mean site velocities (measured within 15 cm of the substrate surface, the usual position of station-holding trout). This suggests a selection for lower water velocities where the energetic costs of holding position would be low.

During winter, 0+ trout occupied shallower depths and slower water velocities than older trout and this was at least partly related to size. That is, larger (and older) trout were able to occupy positions in deeper, faster water. On the West Coast, age 1+ coho salmon and steelhead trout were found in deeper water in the winter than 0+ fish of either species (Bustard and Narver 1975a). Similar size-related habitat preferences have also been noted for salmonids during the summer months (Chapman and Bjornn 1969), apparently a function of changing environmental requirements with growth (Chapman 1966) and energetic considerations related to body size (Smith and Li 1983). Cunjak and Green (1983) found 0+ brook and rainbow trout occupying slower and shallower water than older trout in summer and noted that the degree of difference was related to stream morphology. In the present study, site characteristics also affected the microhabitats selected by different age groups of individual brook and brown trout. Where the range of water flows (or depths) at a site was high, a more obvious relationship with fish size was demonstrated, perhaps a consequence of greater habitat diversity reducing niche overlap.

Cover association is a complex phenomenon. Bustard and Narver (1975b) found that juvenile coho salmon and cutthroat trout (*Salmo clarki*) preferred experimental channels with overhead bank cover rather than open channels whereas Ruggles (1966) found that the carrying capacity for juvenile coho salmon was actually reduced in stream channels where cover (concrete blocks) was present; this result indicated that a species' response can vary with the type of cover present. Bustard and Narver (1975a) observed that 0+ steelhead overwintered beneath rubble (unlike coho salmon) and that the use of rubble shelters by different ages was dependent on the availability of suitable-sized substrates. Similar substrate-hiding in winter has been described for Atlantic salmon (*Salmo salar*) parr (Smirnov et al. 1976; Rimmer et al. 1983) and brook trout in northern Quebec (Cunjak and Power, unpubl. data) and Newfoundland (Cunjak 1982). In the Credit River, brook trout (or brown trout) were never found overwintering beneath rocks, perhaps indicating a latitudinal cline to the behaviour. At the northern limit of its distribution, where winters are characteristically severe and streams often freeze completely, overwintering within the substrate would be adaptive for juvenile

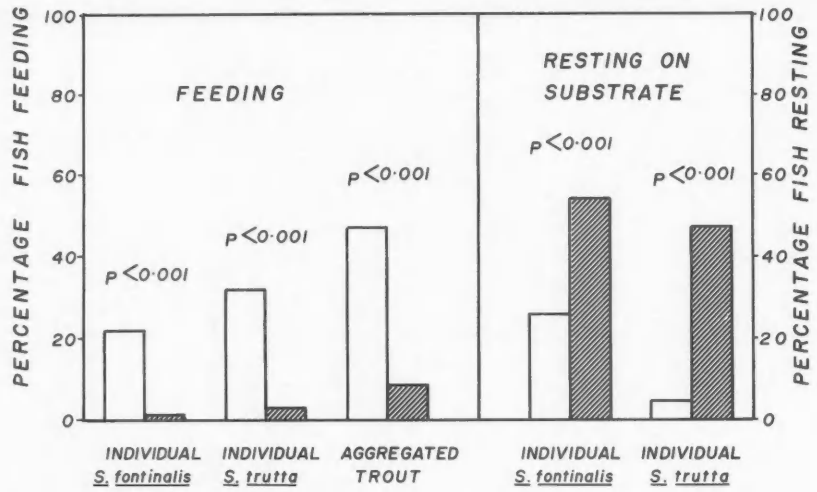


FIG. 4. Percentages of individual and aggregated trout observed making feeding attempts (left panel) and resting on the substrate (right panel) during summer (open bars) and winter (shaded bars). Probability values are for interseasonal comparisons. Data for all sites were pooled for the analyses.

salmonids which deplete their lipid reserves disproportionately faster than do older fish (Mason 1976). Farther south, a trout stream in winter experiences alternating freezing and thawing, variable discharge, and three types of ice formation. In such conditions, an active existence may be the most adaptive strategy for overwintering (Reimers 1963) although competition for winter shelters might also influence which particular behaviour is adopted. In the Credit River, blacknose dace (Cunjak and Power 1986a) and other cyprinids overwintered beneath rocks as did crayfish and frogs (Cunjak 1986).

In winter, water depth may be most important where it affords sufficient cover and/or protection from current rather than being a primary criterion for habitat selection. For instance, at the Caledon site, many 0+ brook trout overwintered in the shallow water of a stream braid where abundant cover (branches) and low flow conditions apparently satisfied the species' winter habitat requirements regardless of depth. Such an anomaly further emphasizes the importance of site-specific influences in affecting habitat selection.

That significant seasonal differences for habitat variables were not realized more often in our study may be explained by species-specific habitat preferences. Brook and brown trout are primarily pool-dwellers in summer (Nyman 1970), and since pools and slow stream reaches are also the main winter habitats, seasonal differences may not be as discernible. Bustard and Narver (1975a) used such an argument to explain similar focal point velocities of juvenile coho salmon (a pool-dweller) between seasons.

Interspecific comparisons of winter habitat utilization at the two sites of sympatry indicate considerable overlap. Nyman (1970) and Fausch and White (1981) found that brook and brown trout have similar ecological demands, with brown trout usually dominating, often displacing brook trout to less optimal stream habitats. The numerical dominance of brown trout at the North Branch is probably a consequence of such competitive superiority and encroachment by this species since its introduction. Upstream of Cataract (only 1 km above the North Branch site), brook trout are abundant in the absence of brown trout. The scarcity of brown trout at the Spring Tributary is probably due to an avoidance of such small sources of undiluted groundwater (Hansen 1975), even for spawning (Witzel and MacCrimmon 1983), although they are used extensively by brook trout.

To what extent (if any) competition for similar winter habitats affects the observed distributions of brook and brown trout is now known and deserves further attention. The probability of interspecific competition in the Spring Tributary is low although the greater number of brown trout observed there in winter indicates that there is some immigration into the tributary. However, the availability of abundant instream cover, low flow conditions, and the lack of agonistic behaviour at low temperatures should reduce the possibilities of interaction. In the North Branch, the potential for interspecific competition seems equally low, as the relatively few brook trout (all ages) which occur there are usually associated with aggregations or situated near spring-fed tributaries. Brown trout, especially the overyearlings which are more solitary, remain in main river pools.

The gregarious behaviour of brook and brown trout was observed in both seasons but particularly in winter. When both species were involved, brook trout predominated, comprising at least 85% of the aggregations. Winter aggregations have been identified for cyprinids (Moshenko and Gee 1973), centrarchids (Munther 1970), catostomids (Cunjak, pers. obs.), as well as salmonids (Hartman 1965; Mundie and Taber 1983). The behaviour appears to be influenced by a complex of factors including fish density, local physical and hydrological conditions, and environmental factors. Water temperature plays a role, as shown by the increase in group size with declining temperatures. Possibly, as water temperatures decline below a critical threshold, fish abandon feeding positions (to conserve energy and since nutritional requirements are less) and move to winter habitats. This congregation increases local density (especially if stream space is reduced by ice encroachment and low water) which encourages "schooling" behaviour in salmonids (Kalleberg 1958). The theory is supported by the

recognized breakdown in territoriality and decreased aggression at low water temperatures (Hartman 1965), both of which are requisite to formation of aggregations. The size of aggregations may also be a function of habitat structure which would explain why, at the North Branch where pools were scarcest, the groups were largest.

The selected winter habitat of aggregated trout was similar to that of individual trout. Areas of low water velocity and good cover were chosen. Cover was used less in summer at the Spring Tributary and Caledon sites because feeding stations were in faster flows where cover was less available. At the North Branch, aggregated trout were apparently able to procure sufficient food (via drift) and cover at the mouths of spring-fed tributaries or groundwater seeps, in both seasons, as this is where they were almost exclusively found.

The frequency of aggregations near spring sources and the high density of trout at the Spring Tributary attest to the importance of groundwater to salmonid productivity and stream ecosystems, in general (Hynes 1983). Craig and Poulin (1975) found entire stream populations of grayling (*Thymallus arcticus*) and arctic char (*Salvelinus alpinus*) overwintering in two perennial spring sections of an Alaskan river when the remainder of the system was frozen. The relatively high water temperatures of "winter warm" water (Hynes 1970) would permit trout to maintain metabolic rates closer to their optima and also preclude ice formation thereby protecting trout from ice damage and its associated conditions (Maciolek and Needham 1952). Groundwater can also be important in the summer. Gibson (1966) observed brook trout and Atlantic salmon congregating in areas of cool spring seepage when stream temperatures exceeded 20°C, and King (1937) found over 100 rainbow trout aggregated near a spring source in a Tennessee stream which had warmed to 29°C. These spring-fed tributaries and seeps probably act as "thermal refugia" for stream fish during periods of adverse water temperatures (high or low).

The extent of emigration to overwintering areas by Credit River trout can only be inferred from the results, since investigation of their movements was not attempted. At Caledon, we believe that the relative scarcity of suitable cover effected the increase in aggregation size and the apparent emigration of some brook trout downstream to a series of deep pools. Movement into such stream sections or spring-fed tributaries would reduce the probability of displacement and/or damage caused by periodic floods and subsequent ice scour. Where identified, winter movement is normally in a downstream direction (Chapman and Bjornn 1969; Peterson 1982) when temperatures have decreased to between 5.0 and 10°C (Bjornn 1971).

Low numbers of individual trout seen in winter were often balanced by concomitant increases in aggregation size such as at Caledon, and to a lesser extent, at the North Branch. At the Spring Tributary, individual 0+ brown trout were observed at this site only in the winter, apparently immigrating after spending the summer in the main river (where they were abundant). Out-migration is probably negligible from this stream during the winter, by either species. The reduction in 0+ brook trout abundance at the site appears to contradict this assertion, especially since the difference cannot be explained by an increase in aggregation size. Instead, it is believed that the cryptic behaviour of these small individuals in winter resulted in underestimates of abundance. The thick mats of watercress within which they could hide provided very effective cover, as evidenced from periodic sampling of the population (Cunjak, unpubl. data).

Recent research (Smith and Li 1983; Bachman 1984; Fausch 1984) has described habitat utilization in terms of a balancing of benefits (food abundance, high growth rate, energy conservation) and risks (predation, depletion of body stores, weight loss, susceptibility to physical displacement in injury). Bustard and Narver (1975a) and Rimmer et al. (1983, 1984) have similarly equated the overwinter of salmonids to such a balance where position choice afforded a potential "profit" of environmental factors. Profitable positions should, therefore, ensure fitness and, ultimately, survival. It would appear that in winter the selection of a particular habitat is a simpler choice, since food (feeding) plays a minor role relative to the summer when nutritional requirements are higher. At near freezing water temperatures, trout can survive without food for as long as 4 mo (Reimers 1957). This reduced feeding at low water temperatures led Chapman (1966) to describe salmonid population regulators in winter as solely space (habitat) related. Despite appearing simpler, the complexity of adopting a particularly overwintering stategy is still considerable due to an increase in the number of fish simultaneously attempting to acquire a common goal, i.e. suitable winter habitat. In summer, time spent in nonfeeding (i.e. resting) habitats (often similar to winter habitats) was balanced with time spent at feeding stations. In winter, the result is a "clumping" or "squeezing" effect where fish are restricted in their occupation of a limited spatial commodity.

For many stream fishes, the requirements for winter habitat are similar: low water velocities and abundant cover. Behavioural plasticity and evolution have successfully combined to achieve a means of accommodating all resident species' winter needs. Such a situation is seen at the Credit River sites. Many small nonsalmonid species (catostomids, cyprinids, cottids) occupy positions out of the main flow by moving beneath rubble. They do so in a semi-torpid state, minimizing energy consumption, yet they are still capable of active swimming. This is advantageous, since periodic environmental disturbances (e.g. floods) would necessitate escape swimming to avoid injury. Large-sized nonsalmonids (e.g. suckers) move to overwinter in the deepest pools. Brook and brown trout demonstrate different strategies to overwinter successfully which are dependent on local hydrology, stream morphology, and the life stage of the fish. Sources of groundwater, especially sufficient-sized spring-fed tributaries, serve as excellent areas for overwintering of these small salmonids due to the stability of flows and thermal regime in combination with a year-round, prolific growth of macrophytes which satisfies winter cover requirements. The generally small size of the tributaries precludes their use by the largest trout which instead overwinter in deep main river pools. Gregarious behaviour, especially at the mouths of the spring-fed tributaries, affords trout the means of exploiting the attributes of such environments (i.e. abundance of food, stable flows, and "warm" temperatures) as well as the antipredator benefits of "schooling" (Shaw 1962). Where wintering habitats are limited, such congregations of fish share the few suitable stream sections that are available. In conclusion, Credit River trout display the behavioural plasticity necessary to successfully survive the rigours imposed by the winter stream environment and a temperate latitude climate.

Acknowledgements

We thank the many individuals who assisted with the field work but especially T. Hoggarth and G. Cressman. We are grateful to Mr. J.

Imhof of the Ontario Ministry of Natural Resources (OMNR) for his help, particularly in the initial phases of the project. Dr. H. B. N. Hynes kindly reviewed an earlier draft of the manuscript. Financial assistance was provided by a University of Waterloo Scholarship and Canadian National Sportsmen's Fund Conservation Scholarship to R.A.C. and by an NSERC operating grant (A1728) and OMNR contract (302-11) to G.P.

References

BACHMAN, R. A. 1984. Foraging behaviour of free-ranging wild and hatchery brown trout in a stream. Trans. Am. Fish. Soc. 113: 1–32.

BINNS, N. A., AND F. M. EISERMAN. 1979. Quantification of fluvial trout habitat in Wyoming. Trans. Am. Fish. Soc. 108: 215–228.

BJORNN, T. C. 1971. Trout and salmon movements in two Idaho streams as related to temperature, food, stream flow, cover, and population density. Trans. Am. Fish. Soc. 100: 423–438.

BOVEE, K. D. 1978. Probability-of-use criteria for the family Salmonidae. Instream Flow Inf. Pap. No. 4. U.S. Fish Wildl. Serv. 81 p.

1982. A guide to stream habitat analysis using the Instream Flow Incremental Methodology. Instream Flow Inf. Pap. No. 12. U.S. Fish Wildl. Serv. Biol. Serv. Prog. FWS/OBS-82/26.

BUSTARD, D. R., AND D. W. NARVER. 1975a. Aspects of the winter ecology of juvenile coho salmon (*Oncorhynchus kisutch*) and steelhead trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 32: 667–680.

1975b. Preferences of juvenile coho salmon (*Oncorhynchus kisutch*) and cutthroat trout (*Salmo clarki*) relative to simulated alteration of winter habitat. J. Fish. Res. Board Can. 32: 681–687.

CARLANDER, K. D. 1969. Handbook of freshwater fishery biology. Vol. 1. Iowa State University Press, Ames, IA. 752 p.

CHAPMAN, D. W. 1966. Food and space as regulators of salmonid populations in streams. Am. Nat. 100 (913): 345–357.

CHAPMAN, D. W., AND T. C. BJORNN. 1969. Distribution of salmonids in streams, with special reference to food and feeding, p. 153–176. *In* T. G. Northcote [ed.] Symposium on salmon and trout in streams. University of British Columbia, Vancouver, B.C.

COOPER, E. L. 1953. Periodicity of growth and change of condition of brook trout (*Salvelinus fontinalis*) in three Michigan trout streams. Copeia 1953: 107–114.

CRAIG, P. C., AND V. A. POULIN. 1975. Movements and growth of arctic grayling (*Thymallus arcticus*) and juvenile arctic char (*Salvelinus alpinus*) in a small arctic stream, Alaska. J. Fish. Res. Board Can. 32: 689–697.

CUNJAK, R. A. 1982. Habitat utilization and the behavioural ecology of rainbow trout (*Salmo gairdneri* Richardson) and brook char (*Salvelinus fontinalis* (Mitchill)) in Avalon Peninsula streams. M.Sc. thesis, Memorial University of Newfoundland, St. John's, Nfld. 189 p.

1986. Winter habitat of northern leopard frogs, *Rana pipiens*, in a southern Ontario stream. Can. J. Zool. 64: 255–257.

CUNJAK, R. A., AND J. M. GREEN. 1983. Habitat utilization by brook char (*Salvelinus fontinalis*) and rainbow trout (*Salmo gairdneri*) in Newfoundland streams. Can. J. Zool. 61: 1214–1219.

CUNJAK, R. A., AND G. POWER. 1986a. Winter biology of the blacknose dace, *Rhinichthys atratulus*, in a southern Ontario stream. Environ. Biol. Fishes (In press)

1986b. Seasonal changes in the physiology of brook trout, *Salvelinus fontinalis* (Mitchill), in a sub-Arctic river system. J. Fish Biol. 28. (In press)

EDINGTON, J. M. AND L. MOLYNEUX. 1960. Portable water velocity meter. J. Sci. Instrum. 37: 455–457.

EMERY, A. R. 1978. The basis of fish community structure: marine and freshwater comparisons. Environ. Biol. Fishes 3: 33–47.

ENVIRONMENT CANADA. 1985. Water survey of Canada, daily water level and discharge data, 1982–85. Water Resources Branch, Guelph, Ont.

FAUSCH, K. D. 1984. Profitable stream positions for salmonids: relating specific growth rates to net energy gain. Can. J. Zool. 62: 441–451.

FAUSCH, K. D., AND R. J. WHITE. 1981. Competition between brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*) for positions in a Michigan stream. Can. J. Fish. Aquat. Sci. 38: 1220–1227.

GIBSON, R. J. 1966. Some factors influencing the distributions of brook trout and young Atlantic samon. J. Fish. Res. Board Can. 23: 1977–1980.

GRIFFITH, J. S. JR. 1981. Estimation of the age–frequency distribution of stream dwelling trout by underwater observation. Progr. Fish-Cult. 43: 51–53.

HANSEN, E. A. 1975. Some effects of groundwater on brown trout redds. Trans. Am. Fish. Soc. 104: 96–110.

HARTMAN, G. F. 1965. The role of behavior in the ecology and interaction of underyearling coho salmon (*Oncorhynchus kisutch*) and steelhead trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 22: 1035–1081.

HUBBS, C. L., AND M. B. TRAUTMAN. 1935. The need for investigating fish conditions in winter. Trans. Am. Fish. Soc. 65: 51–56.

HYNES, H. B. N. 1970. The ecology of running waters. University of Toronto Press, Downsview, Ont. 555 p.

1983. Groundwater and stream ecology. Hydrobiol. 100: 93–99.

JEARLD, A. JR. 1983. Age determination, p. 301–324. *In* L. A. Nielsen and D. L. Johnson [ed.] Fisheries techniques. American Fisheries Society, Bethesda, MD.

KALLEBERG, H. 1958. Observations in a stream tank of territoriality and competition in juvenile salmon and trout (*Salmo salar* L. and *S. trutta*). Rep. Inst. Freshwater Res. Drottningholm 38: 55–98.

KEENLEYSIDE, M. H. A. 1962. Skin diving observations of Atlantic salmon and brook trout in the Miramichi River, New Brunswick. J. Fish. Res. Board Can. 19: 625–634.

1979. Diversity and adaptation in fish behaviour. Springer-Verlag, Berlin. 208 p.

KING, W. 1937. Notes on the distribution of native speckled and rainbow trout in the streams at Great Smoky Mountains National Park. J. Tenn. Acad. Sci. 12: 351–361.

MACIOLEK, J. A., AND P. R. NEEDHAM. 1952. Ecological effects of winter conditions on trout and trout foods in Convict Creek, California, 1951. Trans. Am. Fish. Soc. 81: 202–217.

MASON, J. C. 1976. Response of underyearling coho salmon to supplemental feeding in a natural stream. J. Wildl. Manage. 40: 775–788.

MORTENSEN, E. 1977. Population, survival, growth and production of trout, *Salmo trutta*, in a small Danish stream. Oikos 28: 9–15.

MOSHENKO, R. W., AND J. H. GEE. 1973. Diet, time, and place of spawning, and environments occupied by creek chub (*Semotilus atromaculatus*) in the Mink River, Manitoba. J. Fish. Res. Board Can. 30: 357–362.

MOYLE, P. B., AND D. M. BALTZ. 1985. Microhabitat use by an assemblage of California stream fishes: developing criteria for instream flow determinations. Trans. Am. Fish. Soc. 114: 695–704.

MUNDIE, J. H., AND R. E. TABER. 1983. Movements of coho salmon (*Oncorhynchus kisutch*) fingerlings in a stream following marking with a vital stain. Can. J. Fish. Aquat. Sci. 40: 1318–1319.

MUNTHER, G. L. 1970. Movement and distribution of smallmouth bass in the Middle Snake River. Trans. Am. Fish. Soc. 99: 44–53.

NORTHCOTE, T. G., AND D. W. WILKIE. 1963. Underwater census of stream fish populations. Trans. Am. Fish. Soc. 92: 146–151.

NYMAN, O. L. 1970. Ecological interaction of brown trout and brook trout in a stream. Can. Field-Nat. 84: 343–350.

ORTH, D. J. 1983. Aquatic habitat measurements, p. 61–84. *In* L. A. Nielson and D. J. Johnson [ed.] Fisheries techniques. American Fisheries Society, Bethesda, MD.

PETERSON, N. P. 1982. Immigration of juvenile coho salmon (*Oncorhynchus kisutch*) into riverine ponds. Can. J. Fish. Aquat. Sci. 39: 1308–1310.

PORTT, C. B. 1982. A complication of the available data upon which fisheries management decisions for the Credit River watershed may be based. Report to Ontario Ministry of Natural Resources, Toronto, Ont. 95 p.

REIMERS, N. 1957. Some aspects between the relation between stream foods and trout survival. Calif. Fish Game 43: 43–69.

1963. Body condition, water temperature, and overwinter survival of hatchery-reared trout in Convict Creek, California. Trans. Am. Fish. Soc. 92: 39–46.

RIMMER, D. M., U. PAIM, AND R. L. SAUNDERS. 1983. Autumnal habitat shift of juvenile Atlantic salmon (*Salmo salar*) in a small river. Can. J. Fish. Aquat. Sci. 40: 671–680.

1984. Changes in the selection of microhabitat by juvenile Atlantic salmon (*Salmo salar*) at the summer–autumn transition in a small river. Can. J. Fish. Aquat. Sci. 41: 469–475.

RUGGLES, C. P. 1966. Depth and velocity as a factor in stream rearing and production of juvenile coho salmon. Can. Fish Cult. 38: 37–53.

SAS INSTITUTE INC. 1982. SAS user's guide: statistics. SAS Institute Inc., Cary, NC. 584 p.

SHAW, E. 1962. The schooling of fishes. Sci. Am. 206: 128–138.

SMIRNOV, Y. A., Y. A. SHASTOV, AND V. V. KHRENNIKOV. 1976. On the behavior and feeding of juvenile Onega salmon (*Salmo salar* morpha *sebago*) in winter. J. Icthyol. 16: 503–506.

SMITH, J. J., AND H. W. LI. 1983. Energetic factors influencing foraging tactics of juvenile steelhead trout, *Salmo gairdneri*, p. 173–180. *In* D. L. G. Noakes et al. [ed.] Predators and prey in fishes. Dr. W. Junk Publishers, The Hague, The Netherlands.

TSCHAPLINSKI, P. J., AND G. F. HARTMAN. 1983. Winter distribution of juve-

nile coho salmon (*Oncorhynchus kisutch*) before and after logging in Carnation Creek, British Columbia, and some implications for overwinter survival. Can. J. Fish. Aquat. Sci. 40: 452–461.

WITZEL, L. D., AND H. R. MACCRIMMON. 1983. Redd site selection by brook trout and brown trout in southwestern Ontario streams. Trans. Am. Fish. Soc. 112: 760–761.

ZAR, J. H. 1974. Biostatistical analysis. Prentice-Hall, Inc., Englewood Cliffs, NJ. 620 p.

Population Estimates of White Whale, *Delphinapterus leucas*, in James Bay, Eastern Hudson Bay, and Ungava Bay

T. G. Smith and M. O. Hammill

Arctic Biological Station, Department of Fisheries and Oceans, 555 St. Pierre Blvd., Ste. Anne de Bellevue, Que. H9X 3R4

Smith, T. G., and M. O. Hammill. 1986. Population estimates of white whale, *Delphinapterus leucas*, in James Bay, eastern Hudson Bay, and Ungava Bay. Can. J. Fish. Aquat. Sci. 43: 1982–1987.

Coastal reconnaissance and systematic aerial surveys of white whales, *Delphinapterus leucas*, were flown in James Bay, eastern Hudson Bay, and Ungava Bay. In Ungava Bay the numbers of belugas are so low that we are unable to derive population estimates. In James Bay, we estimate 1213 whales with 95% confidence limits of 740–1970 individuals. In eastern Hudson Bay our systematic survey estimates 968 whales (650–1430), with an additional 474 whales counted in traditional areas of coastal concentration. The age structure of whales inshore and offshore appears similar. Our estimates are conservative, since they do not include a correction for animals which might have been underwater as we passed. Since belugas continue to be hunted in Ungava Bay and eastern Hudson Bay, recommendations are made for a conservative management strategy which must soon be implemented.

Des vols de reconnaissance et des recensements aériens systématiques ont été effectués dans la baie James, dans la portion est de la baie d'Hudson et dans la baie d'Ungava, pour évaluer le nombre de bélugas (*Delphinapterus leucas*). Dans la baie d'Ungava, les bélugas sont si peu nombreux qu'il nous est impossible d'en estimer la population. Les recensements systématiques nous ont permis d'estimer que la baie James regroupait 1 213 bélugas avec un intervalle de confiance à 95 % de 740 à 1 970; l'est de la baie d'Hudson en abritait 968 (650–1 430) en plus des 474 animaux recensés aux sites côtiers traditionnels de concentration. Il n'y a pas de différence significative entre la structure d'âge des groupes de bélugas retrouvés dans les zones côtières et celle des groupes du large. Nos estimations sont conservatrices puisqu'elles n'ont pas fait l'objet de correction pour les animaux qui auraient pu être sous l'eau au moment de notre passage. Certaines recommandations sont présentées en vue d'élaborer une stratégie de conservation puisque ces animaux sont encore chassés dans la baie d'Ungava et dans la zone est de la baie d'Hudson.

Received March 6, 1986
Accepted July 3, 1986
(J8709)

Reçu le 6 mars 1986
Accepté le 3 juillet 1986

The white whale or beluga, *Delphinapterus leucas*, inhabits the coastal waters of northern Quebec, from James Bay along eastern Hudson Bay and southern Hudson Strait shores, and throughout Ungava Bay. The summer distribution of belugas suggests that there are two separate populations, one in Ungava Bay and the other in eastern Hudson Bay (Finley et al. 1982). Their consistent re-occupation of estuarine sites such as the Mucalic River (58°16′N, 67°24′W) in southern Ungava Bay and the Nastapoka River (56°54′N, 76°34′W) in eastern Hudson Bay reinforces the suggestion of separate summer stocks. An estimated 9000 belugas are present in winter in Hudson Strait, which appears to be a common wintering area for northern Quebec belugas and for the summer population occupying the southwestern Hudson Bay coastline (Finley et al. 1982).

Nearshore surveys in 1978 and 1980 indicated that the eastern Hudson Bay population might be as low as 160–250 individuals, much reduced from the 6000–7000 or more whales which evidently occupied the area prior to the high commercial harvests in the period from 1854 to 1868 (Breton-Provencher 1980; Finley et al. 1982). In Ungava Bay, summer coastal surveys indicated an even lower population of perhaps less than 50 individuals concentrated around the Mucalic River estuary (Finley et al. 1982).

Our surveys covered the nearshore and offshore areas of the whole of James Bay, most of eastern Hudson Bay offshore to the Belcher Islands (56°12′N, 79°19′W), and the whole of Ungava Bay. We provide minimum and maximum estimates for the two northern Quebec stocks.

Materials and Methods

In late July and early August 1985, coastal reconnaissance surveys and systematic strip-transect surveys were flown at an altitude of 300 m at a speed of 220–260 km/h using a DeHavilland Twin Otter fixed wing aircraft equipped with an Omega VLF navigation system. In the Hudson Bay survey, one observer sat in the copilot's seat and the other sat directly behind the pilot. In the survey of Ungava Bay the observers sat behind the pilot and copilot, one on each side of the aircraft. A 2-km transect width was delimited by placing marks on the windows while flying at survey altitude over measured reference marks on the ground. All data were recorded on microcassettes. Information recorded included number of whales, time of sighting, amount of ice in tenths, the presence of land in tenths, water clarity, and sea state. The data were then transcribed onto survey sheets and for the systematic survey divided into 2′ intervals. All whales seen off transect were also

noted. Concentrations of whales were photographed with a vertically mounted 6 × 6 cm format camera to compare with the visual counts.

Reconnaissance Surveys

Reconnaissance surveys were flown along the coasts except in James Bay (see Fig. 2 and 3) to include all previously known areas of whale concentration (Breton-Provencher 1980). In James Bay, little information existed on the distribution of belugas (Degerbøl and Freuchen 1935; Schwartz 1976; Reeves et al. 1983). Scattered observations of our own suggested the presence of belugas throughout the area, including some winter sightings (Jonkel 1969), but no large summer estuarine concentrations. In eastern Hudson Bay, known areas of aggregations included the Great Whale (55°16′N, 76°50′W), Little Whale (56°00′N, 76°09′W), and Nastapoka rivers and Richmond Gulf (56°15′N, 76°20′W). In Ungava Bay we paid particular attention to Payne Bay (60°03′N, 69°40′W) and the Leaf (59°01′N, 68°56′W), Koksoak (58°33′N, 68°09′W), Mucalic, and George (58°51′N, 66°22′W) rivers.

Systematic Strip-Transect Survey

For the systematic survey, the study area was divided into three strata. The James Bay stratum encompassed all of James Bay lying south of 54°40′N. The Hudson Bay stratum lay between 54°50′N and 58°50′N and extended west from the coast to 81°00′W. The summer distribution of belugas in eastern Hudson Bay was relatively well known because of the number of Inuit villages all along the coast. We did not survey north of Inukjuak, since this area is known to contain few whales during the summer months. The Ungava Bay stratum included all of Ungava Bay lying south of 60°50′N.

The survey coverage in each stratum represented a compromise between a desired level of survey precision and the operating costs. The amount of survey area required to achieve an error coefficient of variation (E) of 10% was calculated by

$$E^2 = \frac{C}{P \times A \times R}$$

where C is the clump factor (a value of 10 was adopted from Smith et al. 1985), A is the stratum area (square kilometres), R is whale density (whales per square kilometre), and P is the proportion of the stratum to be surveyed.

In eastern Hudson Bay, the stratum area was 86 246 km^2. From Breton-Provencher (1980) and Finley et al. (1982), estimates of 250 and 162 animals were obtained for study areas of approximately 2500 and 2970 km^2, respectively, giving densities of 0.1−0.05 whale/km^2. Solving for P gave a survey fraction of 11.6−21.2% ($\bar{X}$ = 16%) required to achieve a 10% level of precision. We applied similar methods to Ungava Bay. There, we used a population estimate of 50 whales from Finley et al. (1982) for an approximate survey area of 4884 km^2. Because of low density for this area, it was not possible to achieve a 10% level of precision; we therefore adopted a desired precision level of 30%. The amount of survey coverage required to achieve this level of precision was 20% for a stratum area of 56 829 km^2. Since no information was available on total numbers of belugas in James Bay, we arbitrarily chose 10% as providing an acceptable level of survey coverage.

In all three strata, transects ran in an east−west direction. In James Bay, transects were evenly spaced at intervals of 10′ of latitude beginning at 51°20′N and ending at 54°40′N (Fig. 1). In Hudson Bay, transects were spaced at intervals of 10′ of latitude between 54°40′N and 55°30′N and between 57°40′N and 58°50′N. We expected that the majority of belugas would be seen near the Nastapoka River. At the same time, we were unsure of the distribution of belugas around the Belcher Islands. Therefore, we reduced the spacing in this core area between 55°30′N and 57°40′N to intervals of 5′ of latitude (Fig. 2). In Ungava Bay, the majority of animals in the summer are hunted in the southern half of the bay, primarily in the estuaries of the Mucalic and Whale rivers. Between 58°20′N and 59°20′N, transects were spaced at intervals of 5′ of latitude. The remaining transects were spaced at 10′ intervals (Fig. 3).

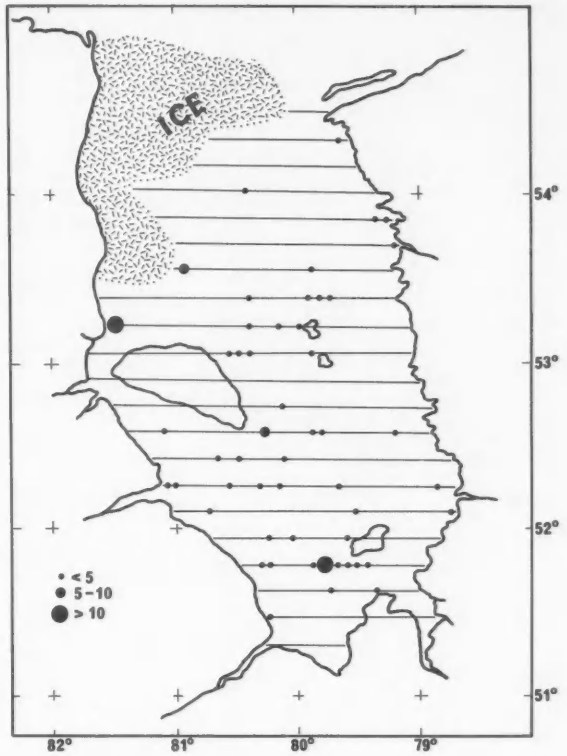


FIG. 1. James Bay survey area showing the systematic survey lines and locations of beluga sightings.

Data Analysis

The design was that of a systematic strip-transect survey. The methods of analysis are as described in Smith et al. (1985) based on Stirling et al. (1977) and Stirling et al. (1982). The present survey was completely systematic, unlike that of Smith et al. (1985) which was based on a random subsample of transects from a systematic survey. Observer counts were tested against each other for significant differences using Student's t-test as in Smith et al. (1985). Multiple regression was used to determine if whale distribution was related to the amount of land or ice or the water depth in each 2′ interval.

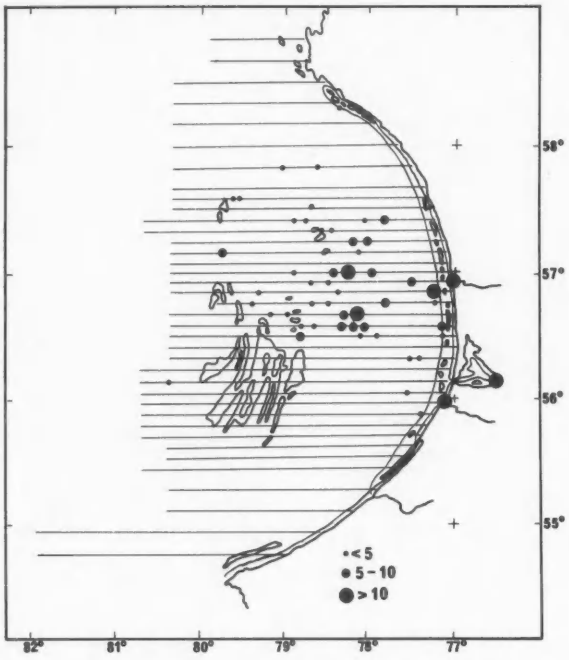


FIG. 2. Eastern Hudson Bay survey area showing the coastal survey route, the offshore systematic survey lines, and locations of beluga sightings.

Information on the presence of land or ice was extracted from the survey notes. Information on water depth for each 2′ interval was obtained from hydrographic charts for the study area.

Other Survey Data

After the completion of our surveys, we gained access to unpublished aerial survey data obtained during the summer and fall of 1982 by M. Allard, Makivik Corporation, 4898 de Maisonneuve, Montreal, Que. These data consist of coastal surveys of Hudson and Ungava bays and a random sample from a systematic strip-transect survey of Ungava Bay.

In Hudson Bay, reconnaissance surveys were flown on 22 September 1982 along the east coast from Inukjuak (58°28′N, 78°10′W) to Great Whale River and back to Inukjuak. A second survey was flown on the same day from Inukjuak to Great Whale River via the Sleeper Islands (57°30′N, 79°55′W) and King George Islands (57°15′N, 78°30′W). A third reconnaissance survey was flown on 23 September from Inukjuak to Cape Jones (62°34′N, 91°51′W).

Reconnaissance surveys of Ungava Bay were flown on 24, 25, and 31 July and 25 August 1982. These surveys concentrated on coastal areas between Stony Point (58°55′N, 68°36′W) and Keglo Bay (59°06′N, 64°46′W) and included the coast of Big Island (58°20′N, 67°35′W) and the Nakoaiyet Islands (58°20′N, 67°30′W).

The strip-transect survey was flown on 26 and 28 September 1982. Transects running north to south were drawn on a map at intervals of 5′ of longitude between 69°30′W and 65°40′W. A total of 24 transects were included in the design of which 15 were selected randomly.

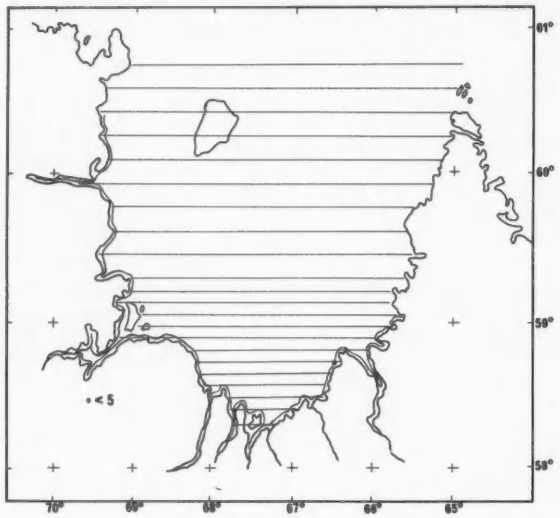


FIG. 3. Ungava Bay survey area showing the coastal survey route, the offshore systematic survey lines, and location of the single sighting of two belugas.

Results

Reconnaissance Surveys

In Hudson Bay, 481 whales were seen during the coastal reconnaissance survey flown on 2 August from Great Whale River to Inukjuak and back (Fig. 2). Based on photographic counts, 48, 287, and 139 animals were seen at Richmond Gulf, Little Whale River, and the Nastapoka River, respectively. An additional three animals were seen at Fraley Island (58°21′N, 78°05′W) and four near McTavish Island (57°33′N, 76°53′W).

In Ungava Bay the coastal survey was flown on 10 August from Dry Bay (60°26′N, 69°38′W) on the west side of Ungava Bay to Kuujjuaq (58°01′N, 68°22′W) and on 11 August from Kuujjuak to Keglo Bay on the eastern side of Ungava Bay. Only two belugas were seen during these flights (Fig. 3).

Systematic Surveys

In James Bay, 21 transects were flown on 3 and 4 August (Fig. 1). Total survey coverage of James Bay was 10% of a total stratum area of 54 779 km^2. In eastern Hudson Bay, 38 transects were flown on 29, 30, and 31 July and 1 and 5 August. Survey coverage was 13.4% of a total stratum area of 56 829 km^2.

During the survey a large portion of the northwest corner of James Bay was covered by very heavy consolidated pack ice. Based on past experience from our High Arctic surveys, such ice contains very few belugas. Therefore, transects over this area ended at the ice edge. Water along the west coast of James Bay was also very turbid from the presence of silt, which may have reduced our counts.

A total of 131 and 205 animals were counted on transects in James and Hudson bays, respectively. No whales were seen in Ungava Bay. Population estimates from the systematic surveys were of the order of 1000 for each of James Bay and Hudson Bay (see details in Table 1).

TABLE 1. Error coefficients of variation (E_1 = variation in whale density over the whole stratum; E_2 = variation in density between adjacent transects), clumping factors (C_1 = clumping factor over the whole stratum measured between adjoining transects), population estimates, and 95% confidence limits (Smith et al. 1985) of belugas in James and eastern Hudson bays.

	E_1	E_2	C_1	C_2	C_1/C_2	Density (whales/km^2)	Population estimate (with 95% confidence limits)	Stratum SE
James Bay	0.239	0.239	7.484	7.498	0.998	0.022	1213 (740–1970)	290
Eastern Hudson Bay	0.270	0.170	14.940	5.931	2.519	0.112	968 (650–1430)	165

The effectiveness of stratification in reducing survey variance can be determined by comparing the C_1/C_2 ratio for the individual strata and for all strata combined. C_1 measures clumping across the whole stratum; C_2 measures the amount of clumping between adjacent transects. A C_1/C_2 ratio close to 1 indicates that whales are dispersed homogeneously across the stratum. James Bay had a C_1/C_2 ratio of 1.0. Hudson Bay had a C_1/C_2 ratio of 2.5, a result of higher numbers of whales being found along transects 17–30 which lay between Sanikiluaq (56°36′N, 79°12′W) in the Belcher Islands and the South Sleeper Islands (57°30′N, 79°45′W) and included transects near the Nastapoka River (Fig. 2). The C_1/C_2 ratio for the unstratified data was 1.5. This value is close to the C_1/C_2 ratios for the individual strata, indicating that stratification was not as effective as desired in reducing survey variance.

Observer bias for the Hudson and James Bay surveys was examined by pooling the data from all the strata and removing any counts where water clarity or the presence of ice or land might have affected the estimates. No significant differences were found between observers ($t = 0.58$, df = 106, $p < 0.05$).

The distribution of whale sightings in the strip-transect survey was examined by dividing the sightings into four classes: less than 10 km from shore, 10–20 km from shore, 20–30 km from shore, and greater than 30 km from shore. In Hudson Bay, 80% of the animals were seen more than 10 km from land; in James Bay, 68% of the animals were sighted more than 10 km from land.

In the stepwise regression analysis, water depth was the only variable found to be significant. The number of whales (Y) was related to water depth (X) by the regression

$$Y = 0.425 - 0.003X \ (F = 10.2, \text{df} = 1,1225, p < 0.01).$$

Other Survey Data

During the reconnaissance surveys flown by Makivik Corporation on 15 July 1982 in eastern Hudson Bay, 339 whales were seen at the following locations: 150 at the Nastapoka River, 45 whales near Mowat Island (56°58′N, 76°40′W), 140 in Richmond Gulf, and 4 at Little Whale River. During the survey conducted on 22 September, six whales were seen near Duck Island (55°45′N, 77°12′W) and one was seen at Richmond Gulf.

In Makivik Corporation's reconnaissance survey of Ungava Bay, whales were sighted as follows: two on 24 July at Whale River; two on 25 July at Big Island, one at Whale River, and one at Keglo Bay; two on 31 July at Whale River, two along the coast west of Alukpaluk Bay (58°23′N, 76°04′W), at the northeast tip, and one in the Tunalic River (58°28′N, 66°50′W); seven on 25 August near Big Island and five in the Whale River although the latter group may have been part of the group of seven seen earlier in the day near Big Island.

In Makivik's offshore strip-transect survey of Ungava Bay, six whales were seen, only one of which was within the 800-m-wide transect. During this survey, three belugas were seen 27 km north of Saeglorsoak Island (58°25′N, 67°30′W) and two were seen 29 km north of Hopes Advance Bay (59°21′N, 69°36′W). No population estimates were calculated from this survey.

Discussion

Our results indicate that more belugas occur in eastern Hudson Bay than previously thought based on coastal counts (Breton-Provencher 1980; Finley et al. 1982). Adding the inshore concentration of 474 belugas to the figures from the systematic surveys yields an estimate of 1124–1904 whales for the eastern Hudson Bay sector. We also estimate that 740–1970 belugas can be found in James Bay during the summer months. This area had never been surveyed, and being contiguous with the eastern Hudson Bay, may be from part of the same stock.

In Ungava Bay the densities of whales are so low that we cannot derive a meaningful population estimate. Our results are supported by the survey conducted by Makivik Corporation in 1982.

Considering the various sources of error inherent in estimating the numbers of animals from aerial surveys (Caughley 1974), we feel that our population estimates are on the conservative side. We did not attempt to estimate the numbers of animals missed because they were underwater. Correction factors for this source of error which are applicable to aerial surveys do not yet exist. Such factors have been used (Sergeant 1973; Brodie 1971; Frost et al. 1985), but these have been obtained from shore-based observations (Brodie 1971) which have different fields of view and observation periods than do aerial surveys. Before correction factors can be applied to aerial survey data, long runs of observational data from aircraft are required, possibly in combination with radio telemetry (Eberhardt et al. 1979; Frost et al. 1985). Separate correction factors may also be required depending on the behavior of the whales, composition of the herd, water depth, and water clarity.

Our estimates for eastern Hudson Bay include the addition of 474 belugas seen in the coastal surveys to the offshore estimates obtained from the systematic counts. We feel this is justified, since our shore-based observations indicate the presence of similar numbers of belugas in the Nastapoka and Richmond Gulf areas throughout the summer period. We com-

TABLE 2. Summary of beluga catches in northern Quebec from DFO/Anguvigak harvest study interviews.

	1981	1982	1983	1984
Eastern Hudson Bay				
Kuujjuarapik	32	45	46	35
Inukjuak	26	18	19	58
Povungnituk[a]	—	—	—	—
Akulivik	3	10	4	4
Southern Hudson Strait				
Ivujivik	58	126	69	69
Salluit	57	41	53	20
Kangiqsujuak	14	21	22	26
Quaqtaq	28	25	38	46
Ungava Bay				
Kangiqsuk	14	9	12	3
Aupaluk	4	2	3	2
Tasiujak	5	6	13	4
Kuujjuaq	30	29	12	5
Kangiqsualujjuak	26	12	3	5

[a]Harvest statistics unavailable; belugas only occasionally obtained in this region.

pared the age structure of inshore and offshore groups based on the number of calves of different length, but found no significant differences. It is possible that there is a continuing exchange between the coastal and offshore whales throughout the summer.

Because of this persistence in returning to nearshore sites, belugas are especially vulnerable to overexploitation. It is at such sites during the summer that more than 50% of the annual beluga catch is taken. At the Nastapoka River where we conducted studies during 1983 and 1984, 8 of 18 individually recognized whales from the first year were resighted there 1 yr later. We observed a number of hunts in the estuary which resulted in the complete abandonment of the site by the whales. The numbers of whales returned to normal within 12–56 h (Caron and Smith 1985). It is evident from this study that belugas are site tenacious from year to year and even over a short period in the face of direct harassment.

The strong evidence of site tenacity supports the concept of discrete stocks defined by their summer distribution and numbers. This strongly suggests that these stocks must be managed on the basis of their estimated summer populations.

The management of belugas is complicated by the fact that another large stock of whales which summers in western Hudson Bay (Sergeant 1973) mixes with those of northern Quebec during the winter in Hudson Strait and Ungava Bay. During that period it is not known what proportion of the catch comes from the northern Quebec stocks.

The calculation of sustainable yields for beluga populations should be based on mortality rates calculated from unbiased age structures and age-specific reproductive rates. Unfortunately, representative age structures from hunted animals are difficult to obtain because of hunter selection of certain age classes and social segregation among the whales. Sergeant (1973, 1981) and Sergeant and Brodie (1975), on the basis of reproductive rates and harvest levels during the commercial hunt from 1955 to 1964 in western Hudson Bay, suggested that beluga populations would sustain catches at levels of 0.05–0.10 of their population size.

Recent harvest statistics and our present population estimates indicate that current exploitation rates are probably above safe levels for both Ungava Bay and eastern Hudson Bay (Table 2).

A conservative management strategy should now be implemented. Hunting of belugas in Ungava Bay should be terminated and the population in eastern Hudson Bay managed at a safe harvest level. There, a conservative quota of 5% of the minimum estimate of 1124 whales would allow a yield of 56 belugas per summer. Our conservative approach will allow a margin of safety in view of the fact that some whales from the eastern Hudson Bay stock are also likely taken by some of the Hudson Strait communities during the autumn and spring hunts. The unexpected number of whales in James Bay, which might form a part of the eastern Hudson Bay stock, will probably also allow a further margin of safety in the continued exploitation of the eastern Hudson Bay stock.

Studies directed towards monitoring age-specific mortality and reproductive rates should continue in order to provide an adequate data base for population models aimed at evaluating the effectiveness of management strategies. In the future, tagging and radio telemetry methods should be perfected and applied to assess the contributions of contiguous stocks of belugas to local harvests and to provide a basis for updating population estimates and management strategies.

Acknowledgements

We thank Gary Sleno and D. W. Doidge who conducted the surveys and George Horonowitsch, Leslie Rich, and Bobby Baron who assisted as observers. Mark Allard, Makivik Corporation, Montreal, Que., kindly provided the unpublished results of their 1982 surveys. World Wildlife Fund Canada provided financial support for the salaries and field studies of some of the personnel involved. Arthur Mansfield of the Arctic Biological Station and Mimi Breton, DFO, Quebec, provided valuable editorial comments.

References

BRETON-PROVENCHER, M. 1980. Survey of the beluga population in the Poste de la Baleine region (New Quebec). Rep. Int. Whaling Comm., Cambridge, Doc. SC/32/SM16. (Transl. from French by Dep. Fish. Oceans).

BRODIE, P. F. 1971. A reconsideration of aspects of growth, reproduction, and behaviour of the white whale (*Delphinapterus leucas*), with reference to the Cumberland Sound, Baffin Island, population. J. Fish. Res. Board Can. 28: 1309–1318.

CARON, L., AND T. G. SMITH. 1985. Preliminary results on the status and behavior of a hunted herd of belugas (*Delphinapterus leucas*) in eastern Hudson Bay. Abstracts. Sixth Biennial Conference on the Biology of Marine Mammals, Nov. 22–26, 1985, Vancouver, B.C.

CAUGHLEY, G. 1974. Bias in aerial survey. J. Wildl. Manage. 38: 921–933.

DEGERBØL, M., AND P. FREUCHEN. 1935. Mammals. Report of the 5th Thule Expedition 1921–24. Copenhagen: Gyldendalske Boghandel, Nordisk Forlag. 2(4–5): 1–278.

EBERHARDT, L. L., D. G. CHAPMAN, AND J. R. GILBERT. 1979. A review of marine mammal census methods. Wildl. Monogr. 63: 46 p.

FINLEY, K. J., G. W. MILLER, M. ALLARD, R. A. DAVIS, AND C. R. EVANS. 1982. The belugas (*Delphinapterus leucas*) of northern Quebec: distribution, abundance, stock identity, catch history and management. Can. Tech. Rep. Fish. Aquat. Sci. 1123: v + 57 p.

FROST, K., L. F. LOWRY, AND R. R. NELSON. 1985. Radiotagging studies of beluga whales (*Delphinapterus leucas*) in Bristol Bay, Alaska. Mar. Mamm. Sci. 1(3): 191–202.

JONKEL, C. J. 1969. White whales wintering in James Bay. J. Fish. Res. Board Can. 26: 2205–2207.

REEVES, R., E. MITCHELL, A. MANSFIELD, AND M. MCLAUGHLIN. 1983. Distribution and migration of the bowhead whale, *Balaena mysticetus*, in the eastern north American Arctic. Arctic 36(1): 5–64.

SCHWARTZ, F. H. 1976. Inuit land use in the Hudson Bay and James Bay islands, Vol. 1: 115–120. *In* M. M. Freeman [ed.] Inuit land use and occupancy project. Department of Indian and Northern Affairs, Ottawa, Ont.

SERGEANT, D. E. 1973. Biology of white whales (*Delphinapterus leucas*) in western Hudson Bay. J. Fish. Res. Board Can. 30: 1065–1090.

1981. On permissable exploitation rates of Monodontidae. Rep. Int. Whaling Comm. 31 (SC/31/SM6): 583–588.

SERGEANT, D. E., AND P. F. BRODIE. 1975. Identity, abundance, and present status of populations of white whales, *Delphinapterus leucas*, in North America. J. Fish. Res. Board Can. 32: 1047–1054.

SMITH, T. G., M. O. HAMMILL, D. J. BURRAGE, AND G. A. SLENO. 1985. Distribution and abundance of belugas, *Delphinapterus leucas*, and narwhals, *Monodon monoceros*, in the Canadian high Arctic. Can. J. Fish. Aquat. Sci. 42: 676–684.

STIRLING, I., W. R. ARCHIBALD, AND D. DEMASTER. 1977. The distribution and abundance of seals in the eastern Beaufort Sea. J. Fish. Res. Board Can. 39: 976–988.

STIRLING, I., M. KINGSLEY, AND W. CALVERT. 1982. The distribution and abundance of seals in the eastern Beaufort Sea, 1974–79. Can. Wildl. Serv. Occas. Pap. 47: 23 p.

Habitat Utilization and Behavioral Interaction of Juvenile Atlantic Salmon (*Salmo salar*) and Rainbow Trout (*S. gairdneri*) in Tributaries of the White River of Vermont[1]

William E. Hearn[2] and Boyd E. Kynard

Massachusetts Cooperative Fishery Research Unit, 204 Holdsworth Hall, University of Massachusetts, Amherst, MA 01003, USA

Hearn, W. E., and B. E. Kynard. 1986. Habitat utilization and behavioral interaction of juvenile Atlantic salmon (*Salmo salar*) and rainbow trout (*S. gairdneri*) in tributaries of the White River of Vermont. Can. J. Fish. Aquat. Sci. 43: 1988–1998.

Competition for space between stocked juvenile Atlantic salmon, *Salmo salar*, and wild juvenile rainbow trout, *S. gairdneri*, was examined in stream channel experiments, a field experiment, and in field habitat surveys. In stream channels providing riffle and pool habitats, species differed in their distribution both as underyearlings (0+) and as yearlings (1+). Yearling salmon occurred more often in stream channel riffles during trials with 1+ rainbow trout than during trials testing only salmon; trout distributions in the channels were unaffected by salmon. In a field experiment conducted to determine if the stocking of 0+ Atlantic salmon causes the displacement of resident 0+ rainbow trout, salmon fry were stocked at a density of 85 per 100 m^2. Emigration from the stocked zone and an unstocked control zone were then monitored daily with four weirs. The 0+ salmon had no apparent effect on the rate of movement of resident rainbow trout fry. During summer and fall field surveys, 0+ salmon occupied deeper and swifter water than 0+ rainbow trout; as yearlings these species occupied similar habitats. In late fall, segregation by habitat occurred: Atlantic salmon were primarily in riffles; rainbow trout were primarily in pools. We found no evidence of competition between cohorts of underyearlings; however, the niche shift by 1+ salmon in the stream channels suggested that, at times, juveniles of these species will compete for space.

La concurrence pour l'espace entre le saumon de l'Atlantique (*Salmo salar*) juvénile d'élevage et la truite arc-en-ciel (*S. gairdneri*) juvénile sauvage, a été examinée dans des cours d'eau expérimentaux, sur le terrain et lors d'inventaires d'habitats. Dans les cours d'eau offrant à la fois des seuils et des fosses, les espèces se distinguaient par leur répartition, à la fois avant l'âge d'un an (0+) et après (1+). Les saumons de plus d'un an se trouvaient plus souvent dans les fosses lorsqu'ils étaient en présence des truites 1+ que lorsqu'ils étaient seuls; la distribution des truites dans les cours d'eau était inchangée en présence des saumons. Pour déterminer sur le terrain si l'ensemencement avec des saumons 0+ provoque le déplacement des truites 0+ sédentaires, des alevins de saumon furent introduits à une densité de 85 par 100 m^2. L'émigration depuis la zone d'ensemencement et depuis une zone témoin non ensemencée fut par la suite contrôlée quotidiennement au moyen de quatre pêches à fascines. Les saumons 0+ n'avaient aucun effet apparent sur le taux de déplacement des alevins de truite sédentaires. Au cours des inventaires au printemps et à l'automne, les saumons 0+ occupaient des eaux plus profondes et plus rapides que les truites 0+; les poissons 1+ des deux espèces occupaient les mêmes habitats. À la fin de l'automne s'est produite une ségrégation écologique, les saumons fréquentant essentiellement les seuils et les truites, les fosses. Nous n'avons trouvé aucun indice de concurrence entre les cohortes âgées de moins d'un an; cependant, le changement de niche opéré par les saumons 1+ dans les cours d'eau suggère une concurrence pour l'espace entre les juvéniles des deux espèces à certaines époques.

Received June 6, 1985
Accepted June 20, 1986
(J8283)

Reçu le 6 juin 1985
Accepté le 20 juin 1986

Restoration of the Atlantic salmon, *Salmo salar*, to its historical range in New England river systems is creating new areas of sympatry between this species and wild populations of rainbow trout, *Salmo gairdneri*. An introduced species in eastern North American, rainbow trout occur in Atlantic salmon streams in Newfoundland (Chadwick and Bruce 1981), and they are established in a few coastal streams in New Brunswick (MacCrimmon 1971). In New England, rainbow trout are abundant in several Vermont streams, including the White River watershed, a system containing extensive Atlantic salmon nursery habitat.

The ecological niches of juvenile rainbow trout and Atlantic salmon are similiar; each prefers the riffle habitat of steep gradient, coldwater streams (Keenleyside 1962; Hartman 1965; Gibson 1966; Everest and Chapman 1972). During juvenile stages, food habits of these species are similar; each is a generalist, feeding largely upon invertebrate drift (Ware 1971; Wankowski and Thorpe 1979; Kwain 1983).

Biologists evaluating the success of stocking Atlantic salmon fry in Vermont streams have stated that the habitat occupied by these species is "essentially identical" (Gersmehl and Darrow 1977). Because of their similarities, concern exists that com-

[1]Contribution 99 of the Massachusetts Cooperative Fishery Research Unit.

[2]Current address: Normandeau Associates, Bedford, NH 03101, USA.

petition, if it exists, may lead to declines in rainbow trout populations or salmon smolt production (Everhart 1966; Mairs 1968; Gibson 1981).

Salmonid species are usually territorial during their stream residence; individuals readily engage in agonistic bouts with similar-sized members of other salmonid species. Territoriality is the principal mechanism for regulating population density in salmonids (LeCren 1973; Mortensen 1979). Chapman (1966) stated that, within this family, competition for space substitutes for direct competition for food, cover, or other resources.

Interspecific competition may lead to the partitioning of the available habitat, a process that facilitates coexistence. Investigators have examined competitive relations between steelhead, *S. gairdneri*, and coho salmon, *Oncorhynchus kisutch* (Hartman 1965; Allee 1982); brook trout, *Salvelinus fontinalis*, and brown trout, *Salmo trutta* (Fausch and White 1981); Atlantic salmon and brook trout (Keenleyside 1962; Gibson 1966, 1977, 1981; Randall 1982); steelhead and chinook salmon, *Oncorhynchus tshawytscha* (Lister and Genoe 1970; Stein et al. 1972); and brook trout and cutthroat trout, *Salmo clarki* (Griffith 1972). Juveniles of these species often segregate with respect to stream depth, water velocity, cover, or combinations of these variables.

The objectives of this study were to determine (1) if interspecific interactions between juvenile rainbow trout and Atlantic salmon in a stream channel environment cause a niche shift in either of these species, (2) if the stocking of salmon fry, at densities commonly used in the White River Restoration Program, causes a displacement of juvenile rainbow trout, and (3) by field surveys, if underyearling and yearling Atlantic salmon and rainbow trout differ in habitat use. This study focused upon interspecific competition for space; other aspects of competition such as competition for food or the effects of competition upon population biomass were not examined.

Study Areas

The study areas were in Locust Creek and Stony Brook, two tributaries of the White River in Vermont (Fig. 1). The mainstem of the White River is 80.5 km long and drains an area of 1839 km^2. The only U.S. Geological Survey gauging station in this drainage is in West Hartford, VT, near the White River's confluence with the Connecticut River. Annual discharge in the river during the study period, which extended from April 1981 to December 1983, averaged 38 $m^3 \cdot s^{-1}$ and ranged from a spring maximum of 484 $m^3 \cdot s^{-1}$ to a minimum of 2.9 $m^3 \cdot s^{-1}$ in September 1982.

Originating in Barnard, VT, at an elevation of 640 m, Locust Creek is 17.7 km long and drains an area of 67 km^2. Its mean gradient is about 18 $m \cdot km^{-1}$, and discharge usually varies between 0.08 and 2.86 $m^3 \cdot s^{-1}$, although spring flows have been estimated in excess of 5.7 $m^3 \cdot s^{-1}$. During the course of this study, peak summer temperatures in the lower reach often reached 23°C; during exceptionally warm periods, water temperatures as high as 27°C were recorded. Resident fishes are rainbow trout, brown trout, brook trout, blacknose dace (*Rhinichthys atratulus*), longnose dace (*R. cataractae*), slimy sculpin (*Cottus cognatus*), and white sucker (*Catostomus commersoni*). Rainbow trout is the most abundant salmonid species and together with the sculpin and dace species represent most of the fish biomass in this stream. Brook trout are primarily in the headwaters and cool tributaries. Limited brown trout production occurs in the lowermost 6 km.

Stony Brook enters the White River 8.4 km upstream from Locust Creek; it is 12 km long and drains an area of 47 km^2. It originates in Sherburne, VT, at an elevation of 490 m; mean gradient within the study area was 22 $m \cdot km^{-1}$. Discharge and temperature regime were approximately the same as in Locust Creek, and resident fish species were identical. In 1973 and 1976 the U.S. Army Corps of Engineers dredged much of Stony Brook, creating a high riffle to pool ratio (>10:1) in the study area.

Materials and Methods

Laboratory Studies

We examined the behavioral interactions and microhabitat preferences of juvenile rainbow trout and Atlantic salmon from June to September, 1981–83, using two stream channels located at the White River National Fish Hatchery in Bethel, VT. Channels were designed to provide substrate and flow conditions similar to those used by salmon and rainbow trout in Locust Creek and Stony Brook. The two stream channels were identical; each was 5 m long × 0.6 m wide × 0.6 m high and provided depths ranging from 15 to 50 cm and a current velocity ranging from 0 to 45 $cm \cdot s^{-1}$. The upstream portion contained a 1.5-m-long riffle with a 35- to 50-$cm \cdot s^{-1}$ surface current and 15-cm depth; a pool 50 cm deep was present in the center of each channel; the downstream 2-m section contained both a small pool (50 cm maximum depth) and an area of intermediate depth (28 cm) and surface velocity (12 $cm \cdot s^{-1}$). Eight windows were located along the side of each channel; channel sections were designated as either riffle, pool, or intermediate habitats. Water for the stream channels was continuously pumped from the White River to the headbox in aquarium A. Water flowed from the headbox through channel A and then into and through channel B. After exiting B, the water was partly recirculated back to the headbox by two pumps. Flow through the channels was approximately 21.5 $L \cdot s^{-1}$. Screened partitions at each end of each channel kept fish within their respective channels and excluded them from headbox areas. The substrate consisted of mixed gravel and rubble (collected from local streams) in riffle areas and sand with some rubble in pool areas. Cavities under and between stones provided cover in each area of the channels.

Fish were fed a simulated invertebrate drift consisting of thawed frozen brine shrimp (*Artemia*) that were periodically dispensed from automatic feeders. For 3-minute intervals, about 25–35 brine shrimp were individually siphoned into the headboxes of each channel. These 3-min intervals were spaced every 20 minutes for about 4-h periods after which time the feeders were empty. Feeders were filled with brine shrimp twice daily. The quantity of feed dispensed was adjusted to avoid satiation of the fish.

Blinds constructed of black polyethylene sheets over a wooden frame extended along the length of each channel. "Slit" windows in the blinds afforded excellent viewing of all channel areas.

All fish used in the experiments were collected from Locust Creek or Stony Brook by electrofishing. All rainbow trout were wild fish; juvenile salmon originated from fry planted each June in Locust Creek during the 3-yr study. Salmon fry were of Penobscot River (Maine) stock and were progeny of adults that returned to the Connecticut River. No fish was used in more than one trial. Newly captured fish were cold-branded with liquid nitrogen for individual identification, and then held

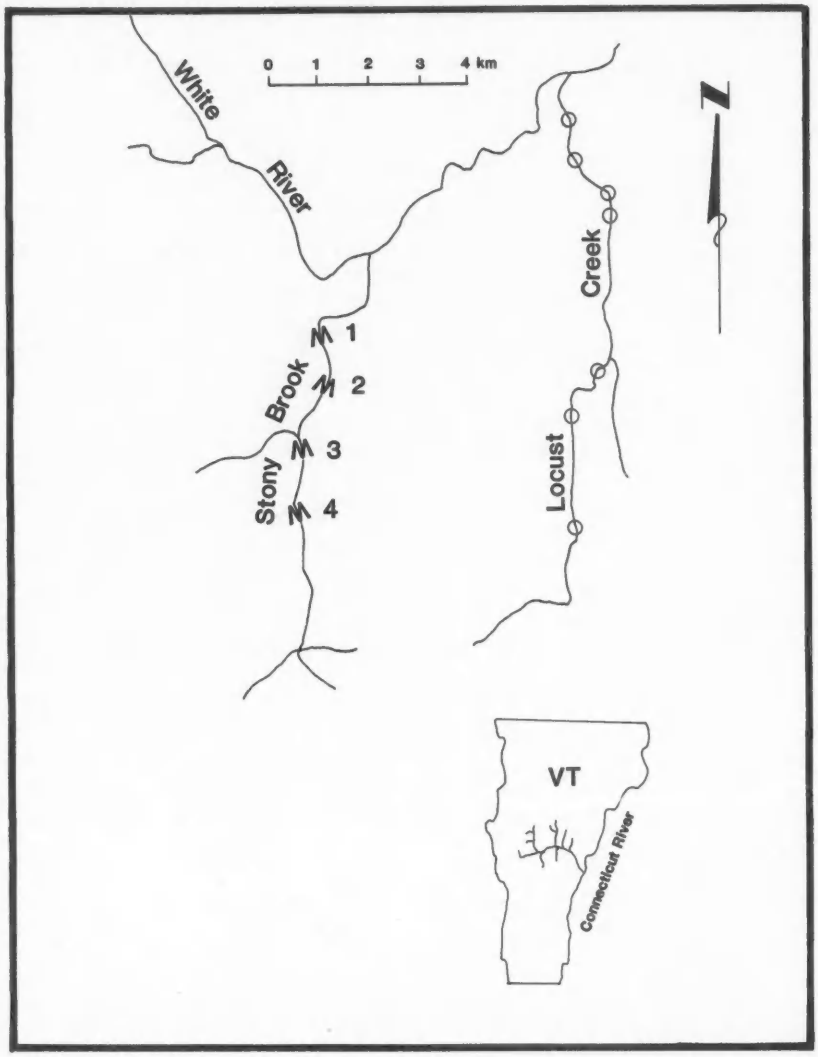


FIG. 1. Study area. Circles on Locust Creek denote sampling stations. Weir sites in Stony Brook are numbered 1–4.

2–6 d in 2300-L circular holding tanks before being used in experiments.

In these experiments, underyearlings (0+) of these species differed in size as they did under local natural conditions. The 0+ salmon were slightly larger than 0+ rainbow trout; yearlings (1+) were of equal size. Size differences between species are detailed later.

Water temperature in the stream channels ranged from 12 to 25°C; however, most data were collected when temperatures were 16–22°C. The habitats selected by both species were not related to water temperature within this range of temperature; however, in early spring when temperatures were below 11°C, both species showed a marked tendency to hide in crevices or beneath stones. Because of this hiding response, trials were not conducted unless water temperatures exceeded 11°C.

We conducted 44 laboratory trials that tested either the agonistic behavior or microhabitat distribution of juveniles within the stream channels (Table 1). Trials varied in the species and ages tested and in the number of fish used. During experiments concerning habitat distribution, densities of fish in the stream channels were comparable with those found in local streams. Trials that quantified agonistic encounters involved densities approximately twice that number. The species were tested both together (interspecific trials) and separately (intraspecific trials). On the first day of a trial, fish were placed into the channels between 14:00 and 16:00. Beginning the next day, for trials concerned with distribution, we plotted the positions of fish two to four times per day with respect to the habitat type and the distance that each fish was positioned above the substrate. Preliminary trials indicated that the general distribution and positions of individual fish varied little after 2 d; therefore, analysis was confined to distributions on days 3 and 4. Multiple

TABLE 1. Laboratory experiments conducted and numbers of fish used in each trial. RT = rainbow trout, AS = Atlantic salmon.

Trial type	Age	No. of trials	No. of fish per trial		Total no. of fish		Fish positions recorded[a]	
			RT	AS	RT	AS	RT	AS
Intra RT	0	4	4		16		96	
Intra RT	0	3	8		24		144	
Intra AS	0	4		9		36		137
Inter	0	6	4	4	24	24	143	139
Intra RT	1	7	3		21		126	
Intra AS	1	8		3		24		141
Inter	1	9	3	3	27	27	158	158
Inter	1	3	6	6	18	18	b	b
Totals		44			130	129	667	575

[a]Total positions recorded on days 3 and 4 of each trial.
[b]Only agonistic behavior was recorded.

observations during these days enabled us to record 301 rainbow trout positions and 297 Atlantic salmon positions during interspecific trials and 366 rainbow trout and 278 salmon positions during intraspecific trials. The agonistic behavior of 0+ fish was recorded during three trials, each of which involved eight fish of each species. The behavior of 1+ fish was similarly examined during three trials, each of which tested six yearlings of each species. During these trials, equal numbers of salmon and trout were individually observed for 6-min intervals, and all charges, chases, nips, lateral displays, frontal displays, intention movements, and threat nips performed by the observed fish were recorded. These agonistic acts have been described by Keenleyside and Yamamoto (1962), Hartman (1965), and Gibson (1977). The agonistic behavior of underyearlings was observed for 1164 min and that of yearlings for 804 min.

We used chi-square contingency tables to examine differences in distribution within the stream channels and species differences in rates of agonistic behavior were tested with chi-square tests.

Field Experiment

From June 27 to October 10, 1982, we conducted an experiment to determine if the stocking of Atlantic salmon fry (0+) causes the displacement of resident 0+ rainbow trout. In June 1982, approximately 4500 salmon fry were stocked at a density of 85 fry per 100 m^2 in a 750-m section of Stony Brook. This area, the "salmon-stocked zone," was bounded with weirs (weirs 1 and 2, Fig. 1) that intercepted emigrating juvenile trout and salmon. Two additional weirs (weirs 3 and 4) monitored movements in and out of a similar-sized control zone, located 1000 m upstream from the salmon-stocked zone. This control zone contained no Atlantic salmon throughout the experiment. Weirs were fence-trap in design, similar to those described by Conlin and Tutty (1979). Downstream traps at each weir collected fish moving in a downstream direction; upstream traps captured fish moving upstream.

The salmon fry were stocked on June 27; the weirs were tended at least once each day from June 28 to September 1 and 4 d per week from September 1 to October 7. Procedures at each weir were identical: the species, age group, previous brands, and lengths of each captured fish were recorded; a combination of pelvic fin clips and liquid nitrogen cold brands was applied to each fish to permit their identification during subsequent recaptures; fish were then returned to the stream and allowed to continue their upstream or downstream movement. In October, after collecting the data, we used the Petersen mark and recapture method to estimate the numbers of juvenile salmon and rainbow trout in the stocked and control zones.

Field Habitat Surveys

From July 1981 to November 1983, we conducted six field surveys to quantify the habitat occupied by juvenile rainbow trout and Atlantic salmon in Locust Creek. A field crew of five or six persons collected data at four 80-m-long stations during each survey trip; in addition, four other stations were irregularly sampled to ensure the capture of adequate numbers of each species and age class (0+ and 1+). Survey procedures at these sites involved the electrofishing of specific types of habitat and emphasized identifying the specific site where a fish was first seen. To minimize the "driving" of fish, we avoided pushing electrodes forward through the water, but placed them down on either side of relatively homogeneous habitat types. Observations by a spotter on the bank indicated that, with this technique, we were not driving fish. At the point where a captured fish was first observed, we recorded the type of substrate, categorized as either silt, sand, gravel (2–50 mm), rubble (50–200 mm), boulder (>200 mm), or bedrock. Water depth at this location was also recorded. In areas with relatively laminar flow, current velocity was measured at the surface and 5 cm above the substrate with a Price pygmy current meter. Velocity measurements were not made in pools or in areas of excessive turbulence. The habitat occupied by fishes of each age class and species was recorded with respect to six habitat categories: shallow riffles (<15 cm deep), deep riffles (>15 cm deep), shallow and deep runs, pools, and turbulent troughs. Shallow runs were 1–15 cm deep and had surface velocities <20 $cm \cdot s^{-1}$; deep runs were 16–50 cm deep and possessed both a surface velocity <45 $cm \cdot s^{-1}$ and a bottom flow <30 $cm \cdot s^{-1}$. Turbulent troughs were defined as turbulent pools (<50 cm deep) located behind or between surrounding boulders; characteristically, water flowed into them from two or more directions. Habitat types were sampled in approximate proportion to their occurrence within the sample stations.

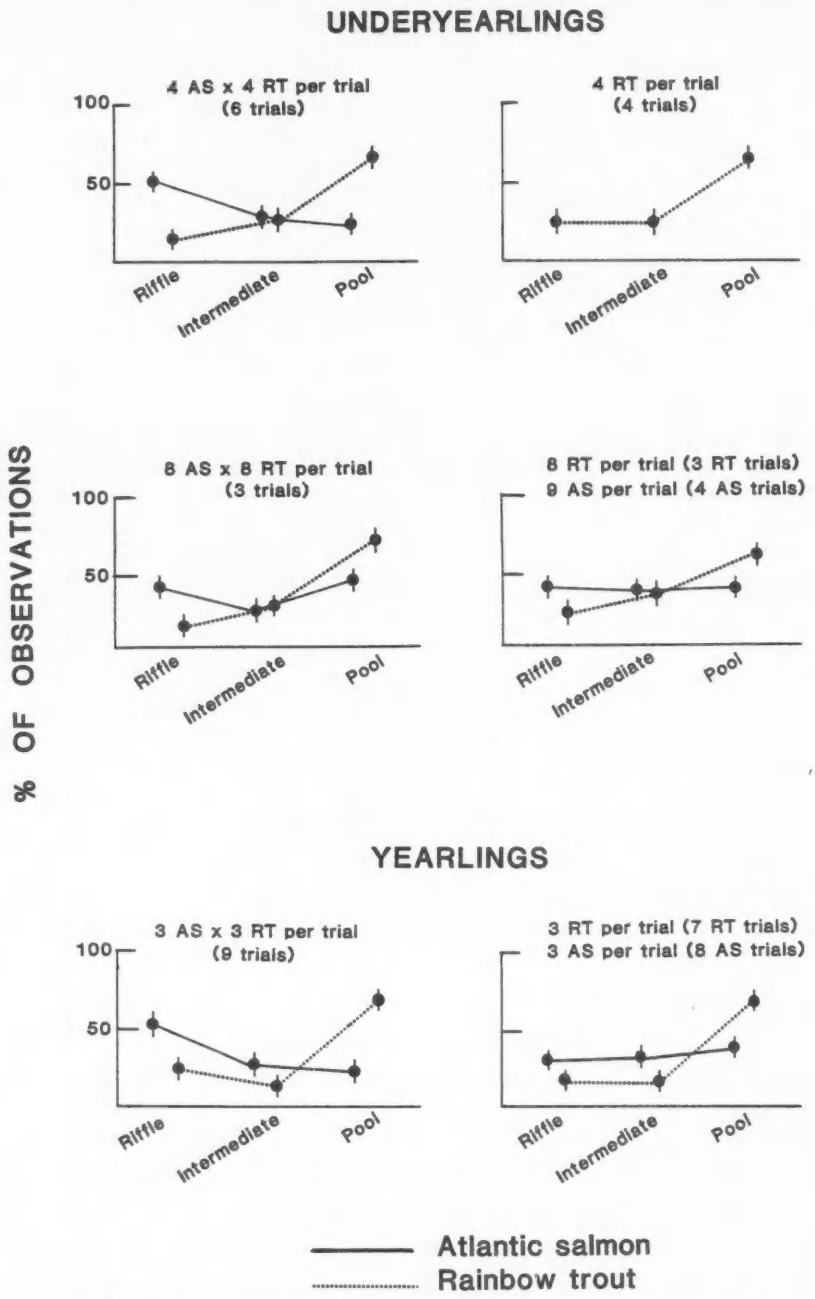


FIG. 2. Distributions of 0+ and 1+ Atlantic salmon (AS) and rainbow trout (RT) in channels during the third and fourth days of each trial. Vertical bars represent SE.

Because stream discharge was not the same during the six surveys, we treated the data from each survey separately. The chi-square test was used to compare the frequency of use of each habitat category by the different cohorts and to test differences in the frequency of substrate categories at capture sites.

Results

Laboratory Experiments

Atlantic salmon and rainbow trout differed significantly in their use of the stream channel habitat during both 0+ and 1+

TABLE 2. Mean number of agonistic acts initiated by individual fish per 6 min of observation. Three 0+ trials tested eight fish of each species and included ninety-seven 6-min observations per species; three 1+ trials tested six fish of each species and included sixty-seven 6-min observations per species. CHG = charge, CHS = chase, IM = intention movement, LD = lateral display, FD = frontal display, THN = threat nip. RT = rainbow trout, AS = Atlantic salmon.

Age and species[a]	CHG	CHS	IM	LD	FD	Nip	THN	Total
Underyearlings								
RT-RT	0.69	0.22	0.42	0.24	0.00	0.12	0.08	1.77
RT-AS	0.15	0.05	0.09	0.01	0.00	0.01	0.00	0.31
AS-AS	0.10	0.01	0.04	0.08	0.01	0.04	0.01	0.29
AS-RT	0.15	0.02	0.09	0.08	0.00	0.02	0.02	0.38
Yearlings								
RT-RT	0.79	0.48	0.45	0.25	0.03	0.18	0.07	2.25
RT-AS	0.34	0.16	0.12	0.07	0.00	0.06	0.07	0.82
AS-AS	0.39	0.10	0.10	0.12	0.06	0.04	0.01	0.82
AS-RT	0.19	0.03	0.12	0.03	0.01	0.01	0.01	0.40

[a]First species is initiator, second species is recipient.

stages, and their distributions differed during both intraspecific and interspecific trials (Fig. 2). During intraspecific trials, 0+ Atlantic salmon were dispersed equally among riffle, pool, and intermediate habitats whereas 0+ rainbow trout were most abundant in the pools ($\chi^2_2 = 10.32$, $p < 0.01$). During two series of interspecific trials, 0+ rainbow trout were again more abundant than salmon in the pools; however, salmon were equally distributed in the riffle, pool, and intermediate habitats only during trials testing eight fish per species. During trials with four fish per species, 0+salmon were primarily in the riffles. During both sets of 0+ interspecific trials, the species differed significantly in their distributions; for trials that tested four 0+ fish per species, $\chi^2_2 = 60.60$, $p < 0.001$; for trials that tested eight fish per species, $\chi^2_2 = 23.43$, $p < 0.001$). During intraspecific trials with yearlings, the species were distributed differently, with trout again primarily in the pools and salmon evenly distributed throughout the stream channels. During interspecific trials, 1+ trout continued to numerically dominate the pools; however, 1+ salmon were significantly more abundant in the riffles ($\chi^2_2 = 53.10$, $p < 0.001$).

A shift in distribution between intraspecific and interspecific trials occurred only among 1+ salmon. Yearling salmon were significantly more abundant in riffles during interspecific trials than during intraspecific trials ($\chi^2_2 = 15.05$, $p < 0.001$) whereas distributions of 1+ trout were unchanged between triai types. During trials with 0+ fish, neither species made a significant shift in habitat use between intraspecific and interspecific trials (χ^2 tests, $p > 0.05$).

The two species also positioned themselves differently in the water column. Rainbow trout invariably maintained positions at least 1−2 cm above the substrate in riffle areas, and in pools they regularly occurred throughout the water column 2−50 cm above the substrate. In contrast, salmon were usually in contact with the substrate; of the 810 recorded salmon positions, less than 10% were off the bottom and each of these was in a low velocity area (<15 cm·s^{-1}).

In trials in which we examined rates of agonistic behavior, rainbow trout initiated three to four times more aggressive encounters than did Atlantic salmon (Table 2). Underyearling rainbow trout directed 85% of their aggressive acts toward other rainbow trout whereas underyearling salmon directed significantly fewer aggressive acts (43%) toward members of their own species ($\chi^2_2 = 46.16$, $p < 0.001$). During trials with yearlings, rainbow trout initiated 72% of all aggressive encounters and directed 73% of their attacks against other rainbow trout; salmon directed 67% of their attacks against other salmon ($\chi^2_1 = 1.31$, $p > 0.05$).

During each observation period, aggression levels varied markedly among individuals. The dominant three to five fish usually performed most of the aggressive acts during a trial; the remaining subordinate fish rarely initiated encounters.

The relative frequency at which specific agonistic displays were performed was about the same for both species. Charging was the most frequent agonistic act performed by either species, and frontal displays were the least frequent (no frontal displays by underyearling rainbow trout were seen).

Field Experiment

Movement of 0+ rainbow trout was largely localized. Of 1007 0+ trout captured, only 34 were recaptures, and among these, 8 were recaptured at the same weir where they were originally marked. Only two 0+ rainbow trout passed over three weirs.

The Petersen population estimates conducted in October 1982 and the collections of fish from the weirs over the 101-d experimental period suggest that 0+ rainbow trout were continuously more abundant in the control zone than in the salmon-stocked zone. The population estimates showed that the density of 0+ rainbow trout was 1.6 times greater in the control zone than in the salmon-stocked zone (Table 3). During the field experiment, 320 0+ rainbow trout were captured as they emigrated from the control zone whereas only 147 were captured leaving the salmon-stocked zone (Table 4).

Because movement was localized, we have assumed that the relative proportions of underyearlings in the control and salmon-stocked zones were approximately constant throughout the field season. The percentage emigration of rainbow trout, expressed as a percentage of the total estimated population, was 7% from the control zone and 5% from the salmon-stocked zone (Table 3). In addition, 364 Atlantic salmon emigrated from the salmon-stocked zone (13% of the total number stocked). Thus, of the 511 0+ salmonids that were captured

TABLE 3. Petersen estimates of underyearling rainbow trout (RT) and Atlantic salmon (AS) in control and stocked zones, total emigration from these zones, and emigration rates expressed as a percentage of the estimated population.

Species	Estimated population (±SE)		Total emigration		Emigration rate	
	Control zone	Stocked zone	Control zone	Stocked zone	Control zone (%)	Stocked zone (%)
RT	4586 (±542) (0.55 fish·m^{-2})	2785 (±369) (0.35 fish·m^{-2})	320	147	7	5
AS	—	2697 (±228) (0.34 fish·m^{-2})	—	364	—	13
Total	4586 (±542)	5482 (±597)		320	7	9

TABLE 4. Total number of underyearling rainbow trout captured at each weir during the period June 28 − October 6, 1982.

Direction of movement at capture	Salmon-stocked zone		Control zone	
	Weir 1	Weir 2	Weir 3	Weir 4
Upstream	14	26	35	29
Downstream	121	210	291	281

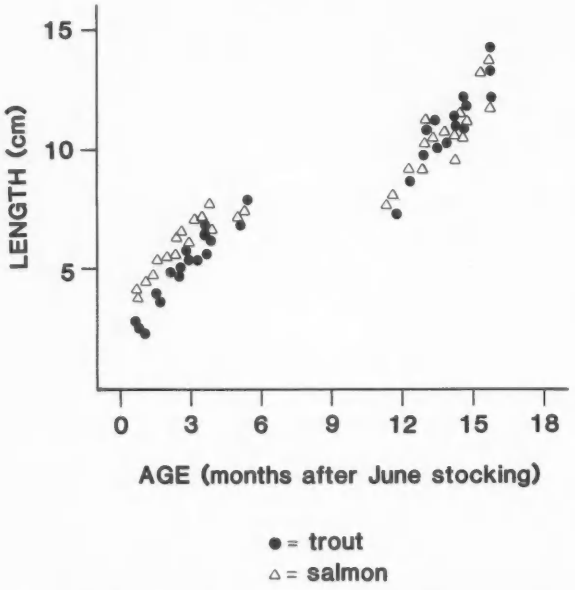


FIG. 3. Total lengths of Atlantic salmon and rainbow trout in Locust Creek and Stony Brook during the first 18 mo following June stockings of salmon fry. Data points are means for daily or weekly collections.

emigrating from the stocked zone, 71% were salmon and 29% were rainbow trout.

During both this field experiment and the field habitat surveys in Locust Creek, 0+ salmon were significantly larger than 0+ rainbow trout until late fall (November), at which time they were equal in size (Fig. 3). Then as yearlings, these species grew at the same rate and were not significantly different in length. Statistical significance was established by separately applying *t*-tests to data for each field survey and to weekly length data collected during the field experiment. The greater size of 0+ salmon is probably related to the relatively late emergence of the spring-spawning rainbow trout and the hatchery conditions experienced by salmon fry prior to their placement in the study streams.

The 0+ salmon and rainbow trout also differed in their direction of movement. At each weir, 89−91% of all captured 0+ rainbow trout were caught in downstream traps; in sharp contrast, more than 98% of all captured salmon were caught in the upstream trap at weir 2, at the upstream end of the salmon-stocked zone.

Periods of peak downstream movement of 0+ rainbow trout differed between weirs. The largest 3-d total captured at a single weir was at weir 1 on July 19−21 (Fig. 4). During this period a significantly smaller percentage of 0+ rainbow trout were captured at the other three weirs ($\chi^2_1 = 29.18, p < 0.001$). Because weir 1 had the only downstream trap that captured emigrants from the salmon-stocked zone, the larger number of rainbow trout captured at this weir during this time may have been due to the presence of the salmon and the associated competition in the stocked zone. Such a conclusion, however, is tenuous, given the variability in the timing and size of movement peaks at the four weirs. At weir 1 on July 28−30 and at weir 3 on August 9−11, downstream movement of 0+ rainbow trout was significantly less than at the other three weirs (chi-square tests, $p < 0.001$). The numbers captured at weir 3 on August 18−20 and in the downstream trap at weir 2 on September 26−28 were significantly greater than the numbers captured at the other three weirs on those dates (chi-square tests $p < 0.001$). These Augusts and September movement peaks were recorded at traps that monitored areas not containing salmon. The factors producing this spatial variability in downstream movement of rainbow trout are unknown; however, there is no good evidence that salmon were responsible. Conditions of stream flow and water temperature may have affected the numbers of 0+ rainbow trout captured in the weirs, although no clear relation could be demonstrated. The peak movement on July 19−21 occurred when water temperatures

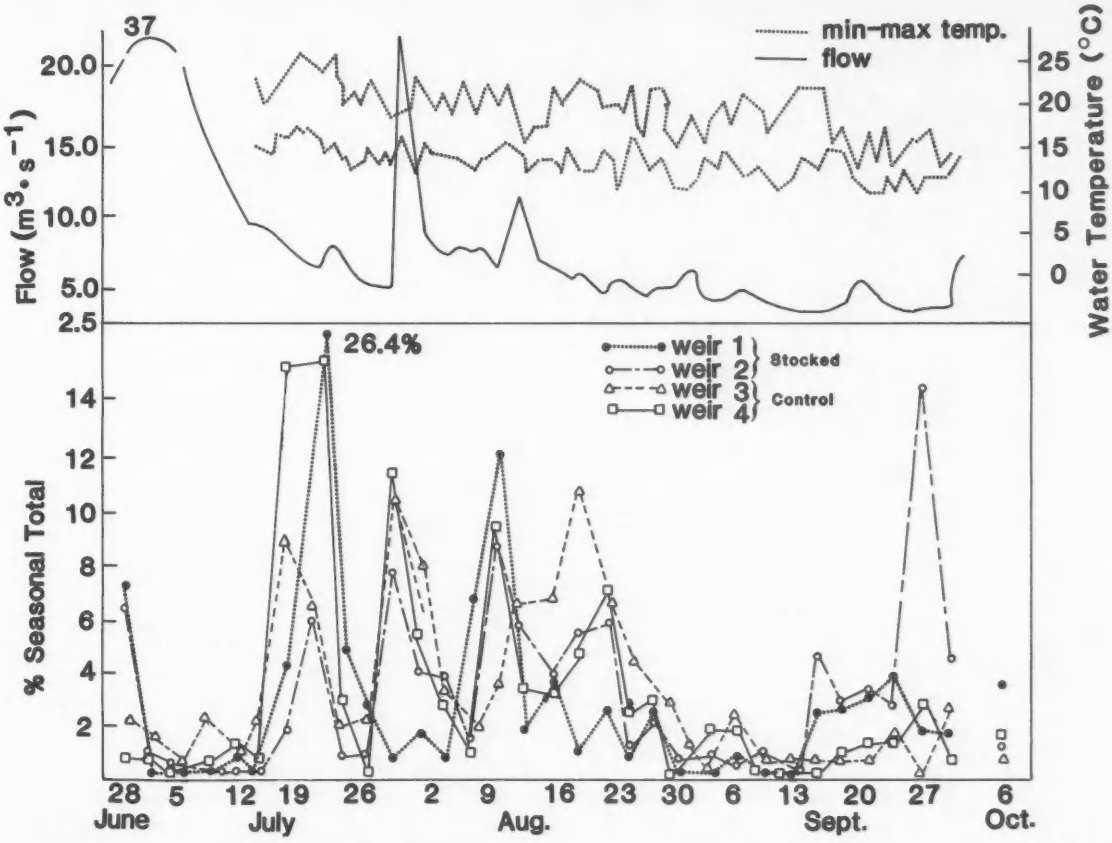


FIG. 4. Daily downstream movement of 0+ rainbow trout during the field experiment and associated Stony Brook water temperatures and White River flows. Weir data are 3-d totals and are percentages of the seasonal total captured at that weir.

were the highest for the season and flows were relatively low (Fig. 4). Peaks on July 29 and August 10 were associated with freshets and average temperatures. During the peak on August 19, temperatures were high and flows were low.

Field Habitat Surveys

In the natural environments of Locust Creek and Stony Brook, juvenile rainbow trout and Atlantic salmon were distributed differently with respect to habitat. During the July 1981 and June 1982 surveys, underyearlings were significantly different in their distribution (Table 5); 0+ salmon occupied swift riffles more often than 0+ trout whereas the latter were more often in pools and shallow runs.

In late June 1982, rainbow trout fry had recently emerged from their redds and were only at depths <17 cm. Consequently, their positions were in significantly shallower water than those occupied by 0+ salmon (Wilcoxon two-sample test, $t_s = 5.35$, $p < 0.001$).

During late September 1982, we observed no differences in the habitats occupied by underyearlings of these species. Yearling salmon and trout occupied the same habitats during summer and early fall surveys, although during all but one of our field surveys, 100% of all 1+ rainbow trout were in riffles >15 cm deep whereas 1+ salmon commonly occupied areas <15 cm deep.

No significant differences between species were found in their distributions relative to the various categories of substrate (chi-square tests). During each survey, boulder or rubble was the principal substrate at the positions of both 0+ and 1+ rainbow trout.

In November 1982 and November 1983, when water temperatures ranged from 4 to 7°C, rainbow trout were concentrated in pool habitats (pools and turbulent troughs) whereas most of the salmon occupied riffles (Table 6).

Discussion

Juvenile Atlantic salmon and rainbow trout occupied dissimilar habitats in Locust Creek and Stony Brook probably as the result of both competitive interactions and innate differences between the species. Evidence that these species prefer different microhabitats are the differences in their distributions during intraspecific trials in the stream channels. Both 0+ and 1+ rainbow trout preferred the deepest areas in the channels whereas, even at low densities, Atlantic salmon occurred equally often in riffle, pools, and intermediate habitats during intraspecific trials. Evidence that competitive interactions may contribute to the segregation of the species is the increased use of riffle habitat by 1+ Atlantic salmon during the interspecific trials.

It cannot be assumed that interspecific competition occurs

TABLE 5. Habitats occupied by juvenile rainbow trout (RT) and Atlantic salmon (AS) at time of capture in Locust Creek. Entries are percentage occurrence in each habitat.

Date				Riffle <15 cm	Riffle >15 cm	Run <15 cm	Run >15 cm	Pool	Turbulent trough	χ^2_6
July 1981	RT	0	39	10	13	18	25	26	8	18.16**
	AS	0	25	64	20	12	4	0	0	
Nov. 1981	RT	0	18	0	17	6	5	61	11	18.36**
	AS	0	29	0	66	0	17	7	10	
June 1982	RT	0	28	0	0	39	0	61	0	13.78*
	AS	0	67	2	12	25	20	38	3	
	RT	1	11	0	46	0	18	0	36	11.20 (ns)
	AS	1	46	0	72	2	15	4	7	
Sept. 1982	RT	0	66	11	19	12	23	18	17	11.74 (ns)
	AS	0	173	14	13	20	30	15	8	
	RT	1	29	0	38	4	31	17	10	8.90 (ns)
	AS	1	38	16	26	5	34	13	5	
July 1983	RT	1	74	7	19	0	31	30	13	10.94 (ns)
	AS	1	108	8	21	6	31	17	17	
Nov. 1983	RT	0	23	4	22	0	17	44	13	35.40***
	AS	0	76	3	76	0	14	4	3	
	RT	1	24	0	8	0	4	88	0	38.15***
	AS	1	50	6	58	0	16	16	4	

TABLE 6. Distribution of rainbow trout and Atlantic salmon in riffles and pools of Locust Creek during early November 1981 and 1983. Data from the two surveys were pooled.

Species	n	Habitat: Riffle	Habitat: Pool	p^a
Underyearling				
Rainbow trout	35	15	20	<0.001
Atlantic salmon	105	95	10	
Yearling				
Rainbow trout	24	3	21	<0.001
Atlantic salmon	50	40	10	

[a]Chi-square test, 1 df.

simply because species overlap in their use of resources. For competition to occur, resource demand must exceed resource availability (Birch 1957). In documenting the niche shift by 1+ Atlantic salmon, this study has developed evidence that juvenile Atlantic salmon and rainbow trout will compete for space.

Because the spatial distribution of 0+ rainbow trout and Atlantic salmon did not differ between intraspecific and interspecific trials (i.e. no niche shift), we developed no evidence that underyearlings of these species compete. Differences in the distribution of 0+ fish in the stream channels and in tributaries of the White River appeared to be due to innate differences between the species, including differences in their emergence time.

Previous studies have reported that juvenile rainbow trout are more aggressive than juvenile Atlantic salmon (Gibson 1981; W. Popp, Cornell Coop. Fishery Research Unit, Ithaca, NY, pers. comm.). In our study, the apparent greater aggressiveness of rainbow trout may have been partly due to interspecific differences in the positions selected within the water column. Positioned on the substrate, Atlantic salmon were more visually isolated than rainbow trout, and therefore less likely to engage in agonistic interactions. Subordinate salmon frequently hid under or among rocks, further decreasing their visibility. In contrast, subordinate rainbow trout were highly visible in their positions located off the bottom.

The increased use of riffles by 1+ Atlantic salmon during interspecific trials appears to be this species' response to competition for space with the more aggressive and pool-oriented rainbow trout. This niche shift was probably facilitated by the wide range of habitats tolerated by juvenile Atlantic salmon. Many studies have reported that juvenile Atlantic salmon regularly use a wide range of habitats including riffles, pools, and even lacustrine habitats (Saunders and Gee 1964; Elson 1967; Pepper 1976; Rimmer and Power 1978). Nevertheless, most studies have indicated that juvenile Atlantic salmon are best adapted to riffles (Gibson 1973, 1981; Jones 1975; Sosiak 1982), and Kalleberg (1958) observed that juvenile Atlantic salmon are more aggressive in riffles than in pools.

The habitats occupied by these species during November field surveys were similar to those reported for allopatric populations. Rimmer et al. (1983) stated that in late fall, when water temperatures are below 10°C, juvenile Atlantic salmon in the Little Sevogle River of New Brunswick remained in riffle and run habitats in proportions similar to those in summer, but that they shift positions from areas above the substrate to spaces beneath streambed stones. We, like Gibson (1978), noted a similar temperature-related hiding response in juvenile Atlantic salmon maintained in stream aquariums. Hartman (1965) and Bustard and Narver (1975) reported that 1+ steelhead prefer deeper pools during winter, although rubble within shallow, low velocity areas provides important winter cover for 0+ rainbow trout.

The results of our field experiment suggest that the salmon fry placed in Stony Brook did not compete intensely with resident 0+ rainbow trout. Evidence supporting this conclusion is as follows. First, the proportion of 0+ rainbow trout that emigrated from the salmon-stocked zone was no greater than that from the control zone. Chapman (1962) and Mason and Chapman (1965) demonstrated that in some salmonid species,

juvenile emigration may result from the displacement of subordinate individuals by dominant territory holders. Competitive interactions have been shown to cause downstream migration in juvenile rainbow trout (Everest 1971; Slaney and Northcote 1974; Erman and Leidy 1975). That no pronounced emigration of rainbow trout occurred even when stocking approximately doubled the number of salmonids in the salmon-stocked zone suggests that the Atlantic salmon had little effect on the 0+ rainbow trout during this experiment. Second, twice as many Atlantic salmon emigrated from the salmon-stocked zone as did 0+ rainbow trout. Symons (1971) reported that juvenile Atlantic salmon may emigrate from areas in response to intense competition for space. If the juvenile salmonids that left the stocking zone were competitively inferior individuals, the greater number of emigrating salmon fry further suggests little impact on resident rainbow trout. Third, about equal numbers of 0+ Atlantic salmon and 0+ rainbow trout were in the salmon-stocked zone 104 d after the salmon fry were stocked. Because instream movement of 0+ rainbow trout was localized, density estimates in October probably represented surviving individuals in each area and not immigrants from other portions of Stony Brook.

It is unknown why the control zone had greater densities of rainbow trout than the stocked zone. Perhaps spawning habitat for rainbow trout was more abundant in the vicinity of the control zone. The greater emigration of 0+ trout from the control zone and the relatively localized movements in and out of the two zones suggest that the lesser density in the stocked zone was not caused by salmon.

Among juvenile salmonids, intensity of competition is generally density-dependent (Egglishaw and Shackley 1977; Gee et al. 1978; Symons 1979). In many populations, when densities are substantially below the carrying capacity of the environment, resources are not limiting and competition may be less intense or nonexistent (Rotenberry and Wiens 1980; Wiens 1983). Therefore, the stocking of Atlantic salmon fry probably leads to greater competition in streams already near their carrying capacity than in streams with less dense populations.

Although Stony Brook's carrying capacity for 0+ rainbow trout is unknown, it appears that their density was relatively high during our study. In October the density of 0+ rainbow trout in the salmon-stocked zone was 35 per 100 m^2. Within other tributaries of the White River, the densities of allopatric 0+ rainbow trout ranged from 4 to 35 per 100 m^2 during 1974–83 (J. Claussen, Vermont Fish and Game Department, Barre, VT 05641, pers. comm.). In Lewis Creek, another Vermont stream, the average density of 0+ steelhead in October was 16 per 100 m^2 (Wentworth 1981).

Prior to the salmon restoration program, juvenile rainbow trout in the White River system were essentially free of competition from other salmonid species, except in tributary headwaters where brook trout are abundant. The niche shift displayed by Atlantic salmon in the stream channels suggests that rainbow trout will sometimes compete for space with Atlantic salmon. If so, this competition probably exerts selective pressures favoring the development of a more clearly defined segregation of habitats (Nilsson 1967). Our experiments suggested that rainbow trout were better adapted to pools and other habitats with low current velocity; their positions off the substrate and greater aggressiveness gave them a competitive advantage over Atlantic salmon in this habitat. In contrast, Atlantic salmon are better adapted morphologically to riffle habitat than trout (Gibson 1981). Atlantic salmon also readily occupy shallow riffle habitat devoid of overhead cover (Gibson 1966; Gibson and Power 1975) whereas in our stream channels, rainbow trout appeared less well-adapted to this microhabitat. Everest and Chapman (1972) reported that in two Idaho streams, most yearling steehead occupied riffles 60–75 cm deep.

The apparent displacement of Atlantic salmon from pool habitats may have implications for fishery managers contemplating the introduction of rainbow trout to Atlantic salmon streams. Interspecific interactions may cause reductions in salmon production, although total displacement from stream sections appears unlikely.

We have collected no information on the competitive relation of these species in deeper riffles (>20 cm) where velocities exceed 35 $cm \cdot s^{-1}$; nor have we information on the effects of interspecific competition on growth rates or population biomass. Gibson (1981) reported that in stream tanks, 45 cm deep and with velocities ranging from 0 to 42 $cm \cdot s^{-1}$, juvenile steelhead preferred moderate currents (17–24 $cm \cdot s^{-1}$) and displaced Atlantic salmon parr from this area into a higher velocity area (40–42 $cm \cdot s^{-1}$). Examination of the interactions of these species in an environment with pools and deep swift riffles or studies examining possible competition effects on population biomass would further facilitate an understanding of the competitive relations of these species.

Acknowledgments

This research was conducted under the auspices of the Massachusetts Cooperative Fishery Research Unit, which is supported by the U.S. Fish and Wildlife Service, Massachusetts Division of Fisheries and Wildlife, Massachusetts Division of Marine Fisheries, and the University of Massachusetts. Principal funding was provided by Region 5 of the U.S. Fish and Wildlife Service. We are grateful to the following persons for their advice and critical manuscript reviews: Drs. J. Gibson, P. Eschmeyer, H. Booke, D. Kroodsma, and M. Ross. Laboratory space and help was generously provided by personnel of the White River National Fish Hatchery. A special thanks to Mr. Paul Gaston, who helped with the design and set up of the stream channels.

References

ALLEE, B. A. 1982. The role of interspecific competition in the distribution of salmonids in streams, p. 111–122. *In* E. L. Brannon and E. O. Salo [ed.] Proceedings of the salmon and trout migratory behavior symposium. University of Washington Press, Seattle, WA.

BIRCH, L. C. 1957. The meanings of competition. Am. Nat. 91: 5–18.

BUSTARD, D. R., AND D. W. NARVER. 1975. Aspects of the winter ecology of juvenile coho salmon (*Oncorhynchus kisutch*) and steelhead trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 32: 667–680.

CHADWICK, E. M. P., AND W. J. BRUCE. 1981. Range extension of steelhead trout (*Salmo gairdneri*) in Newfoundland. Naturaliste Can. 108: 301–303.

CHAPMAN, D. W. 1962. Aggressive behavior in juvenile coho salmon as a cause of emigration. J. Fish. Res. Board Can. 19: 1047–1080.

1966. Food and space as regulators of salmonid populations in streams. Am. Nat. 100: 345–357.

CONLIN, K., AND B. D. TUTTY. 1979. Juvenile salmonid field trapping manual. Fish. Mar. Serv. Can. MS Rep. 1530: 136 p.

EGGLISHAW, H. J., AND P. E. SHACKLEY. 1977. Growth, survival and production of juvenile salmon and trout in Scottish streams, 1966–1975. J. Fish Biol. 11: 647–672.

ELSON, P. F. 1967. Effects on wild young salmon of spraying DDT over New Brunswick forests. J. Fish. Res. Board Can. 24: 731–805.

ERMAN, D. C., AND G. R. LEIDY. 1975. Downstream movement of rainbow trout fry in a tributary of Sagehen Creek, under permanent and intermittent flow. Trans. Am. Fish. Soc. 104: 467–474.

EVEREST, F. H. JR. 1971. An ecological and fish cultural study of summer steelhead in the Rogue River, Oregon. Annu. Prog. Rep. Anadr. Fish Proj. AFS-31. Oregon State Game Commission.

EVEREST, F. H., AND D. W. CHAPMAN. 1972. Habitat selection and spatial interaction by juvenile chinook salmon and steelhead trout in two Idaho streams. J. Fish. Res. Board Can. 29: 91–100.

EVERHART, W. H. 1966. Fishes of Maine. Maine Dep. Inland Fish. Game, Augusta, ME.

FAUSCH, K. D., AND R. J. WHITE. 1981. Competition between brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*) for positions in a Michigan stream. Can. J. Fish Aquat. Sci. 38: 1220–1227.

GEE, A. S., N. J. MILNER, AND R. J. HEMSWORTH. 1978. The effect of density on mortality in juvenile Atlantic salmon. J. Anim. Ecol. 47: 497–505.

GERSMEHL, J. E., AND R. A. DARROW. 1977. Progress report of management studies on juvenile Atlantic salmon in the White River. Fish. Assistance, U.S. Fish Wildl. Serv., Montpelier, VT.

GIBSON, R. J. 1966. Some factors influencing the distributions of brook trout and young Atlantic salmon. J. Fish. Res. Board Can. 23: 1977–1980.

1973. Interactions of juvenile Atlantic salmon and brook trout. Int. Atl. Salmon Found. Spec. Publ. 4: 181–202.

1977. Behavioral interactions between juvenile coho salmon (*Oncorhynchus kisutch*) and juvenile Atlantic salmon (*Salmo salar*) and brook trout (*Salvelinus fontinalis*). ICES C.M. 1977/17:23. 18 p.

1978. The behavior of juvenile Atlantic salmon and brook trout with regard to temperature and to water velocity. Trans. Am. Fish. Soc. 107: 703–712.

1981. Behavioural interactions between coho salmon (*Oncorhynchus kisutch*), Atlantic salmon (*Salmo salar*), brook trout (*Salvelinus fontinalis*) and steelhead trout (*Salmo gairdneri*) at the juvenile fluviatile stages. Can. Tech. Rep. Fish. Aquat. Sci. 1029: 116 p.

GIBSON, R. J., AND G. POWER. 1975. Selection by brook trout (*Salvelinus fontinalis*) and juvenile Atlantic salmon (*Salmo salar*) of shade related to water depth. J. Fish. Res. Board Can. 32: 1652–1656.

GRIFFITH, J. S. JR. 1972. Comparative behavior and habitat utilization of brook trout (*Salvelinus fontinalis*) and cutthroat trout (*Salmo clarki*) in small streams in northern Idaho. J. Fish. Res. Board Can. 29: 265–273.

HARTMAN, G. F. 1965. The role of behavior in the ecology and interaction of underyearling coho salmon and steelhead trout. J. Fish. Res. Board Can. 22: 1035–1081.

JONES, A. N. 1975. A preliminary study of fish segregation in salmon spawning streams. J. Fish Biol. 7: 95–104.

KALLEBERG, H. 1958. Observations in a stream tank of territoriality and competition in juvenile salmon and trout. Rep. Inst. Freshwater Res. Drottningholm 39: 55–98.

KEENLEYSIDE, M. H. A. 1962. Skin-diving observations of Atlantic salmon and brook trout in the Miramichi River, New Brunswick. J. Fish. Res. Board Can. 19: 625–634.

KEENLEYSIDE, M. H. A., AND F. T. YAMAMOTO. 1962. Territorial behavior of juvenile Atlantic salmon. Behaviour 19: 139–169.

KWAIN, W. 1983. Downstream migration, population size, and feeding of juvenile rainbow trout. J. Great Lakes Res. 9: 52–59.

LECREN, E. D. 1973. The population dynamics of young trout in relation to density and territorial behavior. Rapp. P.-V. Reun. Cons. Perm. Int. Explor. Mer 164: 241–246.

LISTER, D. B., AND H. S. GENOE. 1970. Stream habitat utilization by cohabiting underyearlings of chinook (*Oncorhynchus tshawytscha*) and coho salmon (*O. kisutch*) in the Big Qualicum River, British Columbia. J. Fish. Res. Board Can. 27: 1215–1224.

MACCRIMMON, H. R. 1971. World distribution of rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board Can. 28: 663–704.

MAIRS, D. F. 1968. Rainbows downeast. Maine Fish Game. B-158 (Spring 1968), Maine Dep. Inland Fish. Wildl., Augusta, ME.

MASON, J. C., AND D. W. CHAPMAN. 1965. Significance of early emergence, environmental rearing capacity, and behavioral ecology of juvenile coho salmon in stream channels. J. Fish. Res. Board Can. 22: 173–190.

MORTENSEN, E. 1979. Population dynamics, regulation and production of stream salmonids, p. 220–227. *In* The use of ecological variables in environmental monitoring. The National Swedish Environment Protection Board, Rep. PM 1151.

NILSSON, N. A. 1967. Interactive segregation between fish species, p. 295–313. *In* S. D. Gerking [ed.] The biological basis of freshwater fish production. Blackwell, Oxford and Edinburgh.

PEPPER, V. A. 1976. Lacustrine nursery areas for Atlantic salmon in insular Newfoundland. Fish. Mar. Serv. Res. Dev. Tech. Rep. 671: 61 p.

RANDALL, R. G. 1982. Emergence, population densities, and growth of salmon and trout fry in two New Brunswick streams. Can. J. Zool. 60: 2239–2244.

RIMMER, D. M., U. PAIM, AND R. L. SAUNDERS. 1983. Autumnal habitat shift of juvenile Atlantic salmon (*Salmo salar*) in a small river. Can. J. Fish. Aquat. Sci. 40: 671–680.

RIMMER, D. M., AND G. POWER. 1978. Rearing Atlantic salmon (*Salmo salar* L.) in fishless lakes of the Matamek River system, Quebec. Can. Field-Nat. 92: 1–9.

ROTENBERRY, J. T., AND J. A. WIENS. 1980. Habitat structure, patchiness and avian communities in North American steppe vegetation: a multivariate analysis. Ecology 61: 1228–1250.

SAUNDERS, R. L., AND J. H. GEE. 1964. Movements of young Atlantic salmon in a small stream. J. Fish. Res. Board Can. 21: 27–36.

SLANEY, P. A., AND T. G. NORTHCOTE. 1974. Effects of prey abundance on density and territorial behavior of young rainbow trout in laboratory stream channels. J. Fish. Res. Board Can. 31: 1201–1209.

SOSIAK, A. J. 1982. Buoyancy comparisons between juvenile Atlantic salmon and brown trout of wild and hatchery origin. Trans. Am. Fish. Soc. 111: 307–311.

STEIN, R. A., P. E. REIMERS, AND J. D. HALL. 1972. Social interaction between juvenile coho (*Oncorhynchus kisutch*) and fall chinook salmon (*O. tshawytscha*) in Sixes River, Oregon. J. Fish. Res. Board Can. 29: 1737–1748.

SYMONS, P. E. K. 1971. Behavioral adjustment of population density to available food by juvenile Atlantic salmon. J. Anim. Ecol. 40: 569–587.

1979. Estimated escapement of Atlantic salmon (*Salmo salar*) for maximum smolt production in rivers of different productivity. J. Fish. Res. Board Can. 36: 132–140.

WANKOWSKI, J. W., AND J. E. THORPE. 1979. Spatial distribution and feeding in Atlantic salmon. J. Fish Biol. 14: 239–247.

WARE, D. M. 1971. Predation by rainbow trout (*Salmo gairdneri*): the effect of experience. J. Fish. Res. Board Can. 28: 1847–1852.

WENTWORTH, R. S. 1981. Biology of juvenile steelhead rainbow trout and landlocked Atlantic salmon in Lewis Creek, Vermont. M.S. thesis, University of Vermont, Burlington, VT. 94 p.

WIENS, J. A. 1983. Competition or peaceful coexistence. Nat. Hist. 92: 30–34.

Effects of Flooding on Dissolved and Suspended Nutrients in Small Diked Marshes[1]

John A. Kadlec

Department of Fisheries and Wildlife, Utah State University, Logan, UT 84322-5200, USA

Kadlec, J. A. 1986. Effects of flooding on dissolved and suspended nutrients in small diked marshes. Can. J. Fish. Aquat. Sci. 43: 1999–2008.

Flooding ten 5- to 7-ha diked marshes in the Delta Marsh, Manitoba, to about 1 m above natural marsh levels did not increase dissolved or suspended nutrient concentrations in the surface water. Dissolved forms of N and P increased in interstitial water, possibly as a direct or indirect effect of death of emergent macrophytes (e.g. cattail, *Typha* spp.) and associated changes such as wave action and detritus deposition. Concentrations of suspended N, P, and C decreased in surface water as a result of flooding, both in absolute terms and relative to concurrent increases shown by natural marsh controls. Concentrations of major ions (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, SO_4^{2-}, and Cl^{-}) did not change in response to flooding, but did vary in time and space. A predicted decrease in the concentrations of major ions in interstitial water due to seepage of dilute surface water into the sediment was not detected.

Après inondation de 10 marais endigués de 5 à 7 ha dans la région du Delta Marsh, au Manitoba, jusqu'à environ 1 m au-dessus du niveau naturel de l'eau, on n'a pas relevé d'augmentation des concentrations des nutriments dissous ou en suspension dans l'eau de surface. La présence des formes dissoutes de N et P augmentait dans l'eau interstitielle, ce qui peut être un effet direct ou indirect de la mort des macrophytes émergents (p. ex. les massettes, *Typha* spp.) et de changements connexes comme l'action des vagues et le dépôt de détritus. Les concentrations de N, P et C en suspension diminuaient dans l'eau de surface à cause de l'inondation, tant en termes absolus que relativement aux augmentations concurrentes relevées dans les marais témoins. Les concentrations des principaux ions (Ca^{+2}, Mg^{+2}, Na^{+}, K^{+}, SO_4^{-2} et Cl^{-}) ne changeaient pas du fait de l'inondation, mais variaient dans le temps et dans l'espace. On n'a pas pu observer la réduction prévue des concentrations des principaux ions dans l'eau interstitielle à cause de l'infiltration de l'eau superficielle dans le sédiment.

Received November 26, 1985
Accepted June 24, 1986
(J8589)

Reçu le 26 novembre 1985
Accepté le 24 juin 1986

Changes in nutrient availability and dynamics are believed to result from water level fluctuations that are common in both managed (Kadlec 1962) and unmanaged marshes (Weller 1979). van der Valk and Davis (1978) have hypothesized a cyclic pattern of water level and vegetation change in natural prairie marshes. In brief, they proposed that prolonged high water during periods of normal or above normal precipitation led to the creation of a "lake stage" in which emergent vegetation (e.g. cattail, *Typha* spp.) was confined to the marsh margins. They postulated that reestablishment of emergents required a drought to lower water levels and expose the pond bottom. With a return to higher levels of precipitation, the marsh would be reflooded and return to high levels of productivity. Implicit in this proposed cycle are major changes in nutrient concentrations and cycling, but these have not been studied. The studies reported in this paper examine the changes in nutrient concentrations resulting from the experimental creation of the "lake marsh" stage as the first part of a long-term experimental evaluation of the effects of the wet–dry cycle on the major components of wetland ecosystems (Batt et al. 1983).

The effects of flooding to create marshes for waterfowl are similar to those observed when large river impoundments are created (Cook and Powers 1958; Kadlec 1962). A flush of productivity is commonly recorded, presumably as a result of the release of nutrients from inundated vegetation and previously unflooded soils, the reservoir paradigm of Hecky et al. (1981). In the studies reported herein, water depths were increased 1 m and flooded areas doubled compared to the premanipulation marsh states. Consequently, my hypothesis was that the dissolved or suspended nutrients should increase following the increase in water levels. The deep flooding of the experiment reported herein was designed to and did kill most of the existing macrophytes (Murkin 1983), eliminating macrophyte uptake as a potential effect of increased nutrient availability.

Another effect of deep flooding is an increase in seepage loss due to the increased hydraulic head (Kadlec 1983). Because surface waters are usually more dilute (e.g. Kadlec 1982; Ku et al. 1978), my second hypothesis was that concentrations of major ions should decrease in the interstitial water due to increased seepage.

Because the purpose of this study was to examine the foregoing hypothesized effects of flooding with replicated marshes, it was not possible to simultaneously investigate all the mechanisms which might be causes of observed changes. We antici-

[1]Paper No. 8 of the Marsh Ecology Research Program, Delta Waterfowl and Wetlands Research Station – Ducks Unlimited Canada.

TABLE 1. Sequences of growing season water levels in experimental marshes, Marsh Ecology Research Program, Delat Marsh, Manitoba. N = natural level of marsh, about 247.50 ± 0.20 m above sea level, F = 248.41 m, L = 247.50 m, M = 247.80 m, H = 248.10 m.

Treatment[a]		1980	1981	1982	1983	1984	1985	1986	1987
1	Cells 4,8	N	F	F	dry	dry	L	L	L
1	Cells 1,5,9	N	F	F	dry	dry	M	M	M
1	Cells 2,6,10	N	F	F	dry	dry	H	H	H
2	Cells 3,7	N	N	F	F	dry	L	L	L
3	Cells 11,12	N	N	N	N	N	N	N	N

[a]For purposes of this paper, cells 1,2,4,5,6,8,9 and 10 were treated identically and constitute one treatment.

TABLE 2. Overall mean values (SE and *n* in parentheses) for nutrient concentrations for 1980 in the Marsh Ecology Research Program study sites at the Delta Marsh, Manitoba. Dash = not measured.

		Interstitial water		
Constituent	Surface water	15 cm	30 cm	45 cm
pH	7.86 (0.07,106)	7.30 (0.03,237)	7.26 (0.06,46)	7.17 (0.06,47)
Conductivity $\mu S \cdot cm^{-1}$ @ 25°C	3550 (97,78)	7160 (213,179)	9520 (423,41)	10 600 (440,42)
NH_4-N ($\mu g \cdot L^{-1}$)	196 (57,126)	3270 (293,245)	1340 (337,47)	1040 (327,49)
TDN ($\mu g \cdot L^{-1}$)	2990 (178,96)	10 700 (385,245)	8960 (580,47)	8080 (542,49)
SRP ($\mu g \cdot L^{-1}$)	73.9 (17.7,125)	394 (27,244)	131 (28,47)	110 (22,49)
TDP ($\mu g \cdot L^{-1}$)	170 (31,86)	589 (34,245)	247 (35,47)	195 (29,49)
Na ($mg \cdot L^{-1}$)	497 (12,126)	987 (28,243)	1,546 (283,47)	1389 (51,49)
K ($mg \cdot L^{-1}$)	36.0 (0.8,126)	37.6 (1.0,243)	34.5 (1.5,47)	32.2 (1.5,49)
Ca ($mg \cdot L^{-1}$)	69.0 (2.1,126)	188 (4.7,243)	262 (16.0,47)	357 (21.1,49)
Mg ($mg \cdot L^{-1}$)	155 (5,126)	354 (13,242)	485 (30,47)	610 (33,49)
Suspended P ($\mu g \cdot L^{-1}$)	185 (28,120)	—	—	—
Suspended N ($\mu g \cdot L^{-1}$)	757 (115,111)	—	—	—
Suspended C ($\mu g \cdot L^{-1}$)	4150 (515,111)	—	—	—

pated that processes of plant uptake and release (both algae and macrophytes), sorption-desorption, decomposition, chemical precipitation–dissolution, diffusion, mass transport, microbiological N transformations, and others might all be involved (Kadlec 1979). However, only the net result of these processes could be measured within the scope of these studies. Consequently, the objectives of this study were to (1) describe the background conditions as they existed prior to flooding, (2) determine which changes in dissolved and suspended nutrients were due to flooding, and (3) compare those changes with the predicted effects of flooding.

Area Studied

In 1979, the Delta Waterfowl and Wetlands Research Station and Ducks Unlimited Canada initiated a long-term study of the effects of wet–dry cycles on marshes, the Marsh Ecology Research Program, or MERP (Batt et al. 1983). A major objective of MERP is to critically test the concepts of cyclic change in the vegetation of prairie marshes outlined in van der Valk and Davis (1978). For that purpose, a set of 10 contiguous diked 5- to 7-ha marshes (cells) was constructed in 1979 within the large (20 000 ha) Delta marsh about 100 km west of Winnipeg, Manitoba.

The general strategy of the MERP studies is to simulate the wet–dry–wet cycle of natural prairie marshes, utilizing the set of diked marshes to provide replications of the water level treatments. Each treatment is a specific sequence of annual water levels (Table 1). Individual diked marshes (cells) were assigned to 1980–84 treatments at random. The initial high water stages (F in Table 1) were designed to simulate periods of high water that eliminate emergent vegetation, thus initiating

TABLE 3. Average levels (meq·L^{-1}) of major ions and ionic balance based on 1981 overall mean values for cations, Cl^-, and SO_4^{2-} and one set of alkalinity analyses in September 1981.

Cations	
Na^+	20.92
Ca^{2+}	3.49
K^+	0.84
Mg^{2+}	13.12
Total	38.37
Anions	
Cl^-	21.40
SO_4^{2-}	5.47
HCO_3^-	12.24
Total	39.11

the lake stage of a wet–dry cycle. Concurrent studies examined macrophyte, algae, invertebrate, and avian responses to the imposed treatments.

In addition to the 10 experimental marshes, two nearby 6-ha areas of undiked marsh are also being studied. Because the Delta Marsh is contiguous with Lake Manitoba, which is regulated by an outlet dam, water levels in these adjacent areas fluctuate less than unregulated marshes in response to rainfall and drought. Nevertheless, they do undergo some minor (10 cm) short-term changes, especially in response to seiches in Lake Manitoba.

A general description of the marsh and the hydrology of the diked cells is provided by Kadlec (1983). The marsh compartments are separated from the south end of Lake Manitoba by a wooded ridge. The major vegetation types in the natural marsh and the cells are whitetop (*Scholochloa festucacea*), hardstem bulrush (*Scirpus acutus*), cattail (*Typha* spp.), phragmites (*Phragmites australis*), and open-water areas which contained predominantly sago pondweed (*Potamogeton pectinatus*) in 1980.

The growing season of 1980 was used to collect baseline data on each marsh. Consequently, although the dikes were in place, the control structures were open, allowing water levels inside the cells to equilibrate with exterior marsh levels. The correspondence was not precise, but lagged because the limited cross-sectional area of the control structures restricted water movement more than in unmodified marsh.

In late September 1980, pumping was initiated to raise the water level in all diked cells, except 3 and 7, about 1 m by the time of freeze-up, which occurred on 9 November. The flooded cells froze at about 248.3 m above sea level. In early spring 1981, the level of 248.41 m was achieved and maintained by pumping water into the cells whenever the level fell below 248.39 m. In fall 1981, cells 3 and 7 were also flooded to a depth of about 1 m. All 10 diked cells remained deeply flooded (design level 248.41 m) in 1982.

Methods

Surface and interstitial water were sampled monthly during the ice-free season at five permanent random locations in each cell. When the marshes were at normal water levels (Table 1), surface water was not always present at the sample stations and no surface water sample was possible. The sampling wells were made of 5-cm I.D. PVC wells (modified from Neely and Davis 1985) with nylon screened inlets at 15 cm below the sediment–water interface, which represented approximately the middle of rooting zone of the emergent plants. The pipes were permanently sealed at the bottom and at the sediment surface and provided with hose connections for sampling. Water inside the well was at equilibrium with pore water, thus permitting regular sampling without disturbing the sediment. To examine horizontal and vertical variation in more detail, and to evaluate the statistical adequacy of five wells per cell, cell 9 was selected at random and an additional five sample stations established at random (10 total). At all 10 stations, wells were installed to sample interstitial water at 15-, 30-, and 45-cm depths.

Surface water samples were collected by submersing an acid-washed polyethylene bottle, being careful to avoid sediments stirred up by wading. Interstitial water samples were taken by pumping the required volume via hoses permanently attached to each well. Because of the large number of samples, no effort was made to preserve in situ redox potential. Consequently, we expected changes in state of some constituents, notably Fe, S, and forms of P. However, because our objective was to compare treatments, this was not critical. Temperatures of the surface and interstitial water were taken in the field and the samples were returned to the field laboratory where conductivity (YSI model 33 meter), temperature, and pH (Orion model 407A meter) were measured. After filtering aliquots for analyses of dissolved and suspended fractions, all samples were iced and shipped overnight for analysis at the Freshwater Institute (Department of Fisheries and Oceans) in Winnipeg, Manitoba. Critical analyses were performed the next day, i.e. within about 24 h. All analyses were by the methods of Stainton et al. (1977). Analysis of surface water samples included NH_4^+-N, NO_3^--N, total dissolved N (TDN), soluble reactive P (SRP), total dissolved P (TDP), Na^+, K^+, Ca^{2+}, Mg^{2+}, CL^-, SO_4^{2-}, and suspended N, P, and C. Interstitial water samples were analysed only for the dissolved constituents because previous studies (Kadlec 1976) had shown that the well method of sampling interstitial water did not permit sampling of suspended materials in a meaningful way. In 1980 only, analyses for NH_4^+-N, NO_3^--N, TDN, SRP, and TDP were made at the field laboratory.

Statistical analyses were performed using the ANOVA method of SPSS (Nie et al. 1975) on a VAX 11/780 computer. The 0.05 level of alpha was adopted for all tests of significance. In the context of this study, the sample stations within individual marshes (cells) were considered subsamples, with each marsh (cell) being the statistical sample unit. Therefore, following Hurlbert (1984), tests for treatment effects were conducted using the mean values for each cell as the data set.

Results

Preflooding

At normal water levels, the study cells were high in all dissolved constituents and in pH (Table 2). High levels of Na^+, Mg^{2+}, Cl^-, and SO_4^{2-} (Table 3) were the chief causes of the high conductivity and salinity. Interstitial water was lower than surface water in pH, but higher in all dissolved constituents except K^+ at 30 and 45 cm. Conductivity and concentrations of Na^+, Ca^{2+}, and Mg^{2+} of interstitial water increased with greater depth in the sediments. In contrast, concenterations of K^+ and the forms of N and P tended to decrease with increasing depth.

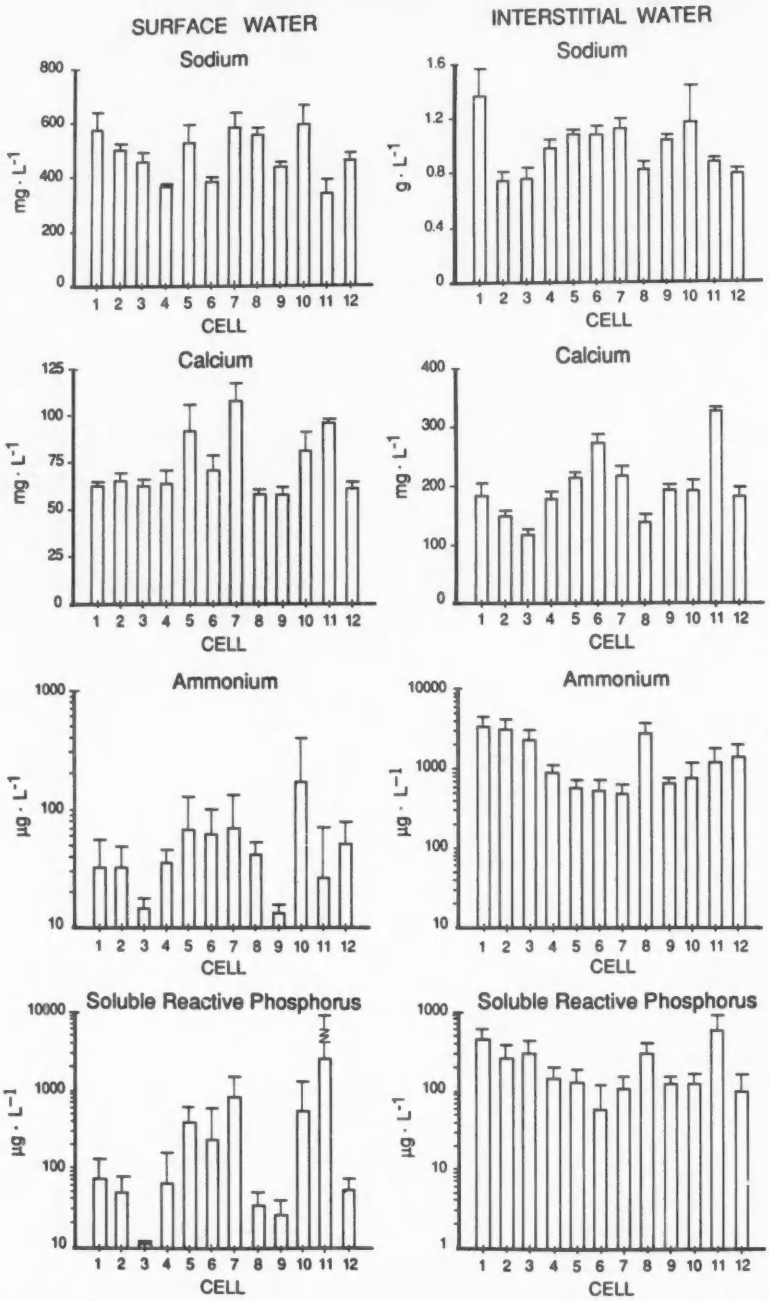


FIG. 1. Variation among cells in concentrations of selected nutrients in surface water and interstitial water in 1980 at natural marsh water levels. Lines represent S.E.

Because water levels in all cells were at natural marsh levels in 1980, it was possible to assess the natural variation in the various constituents in space and time (Fig. 1, 2, and 3). Variability among the cells in concentrations of most ions was high (Fig. 1) and often significant (note small SE in many cases). Interactions between season and cell effects (ANOVA) in interstitial water were not significant, suggesting that all cells followed similar seasonal patterns.

One possible cause of the differences among cells was that the area occupied by different vegetation communities varied from cell to cell. Because the sample stations were located at random, they were also a valid sample of vegetation types,

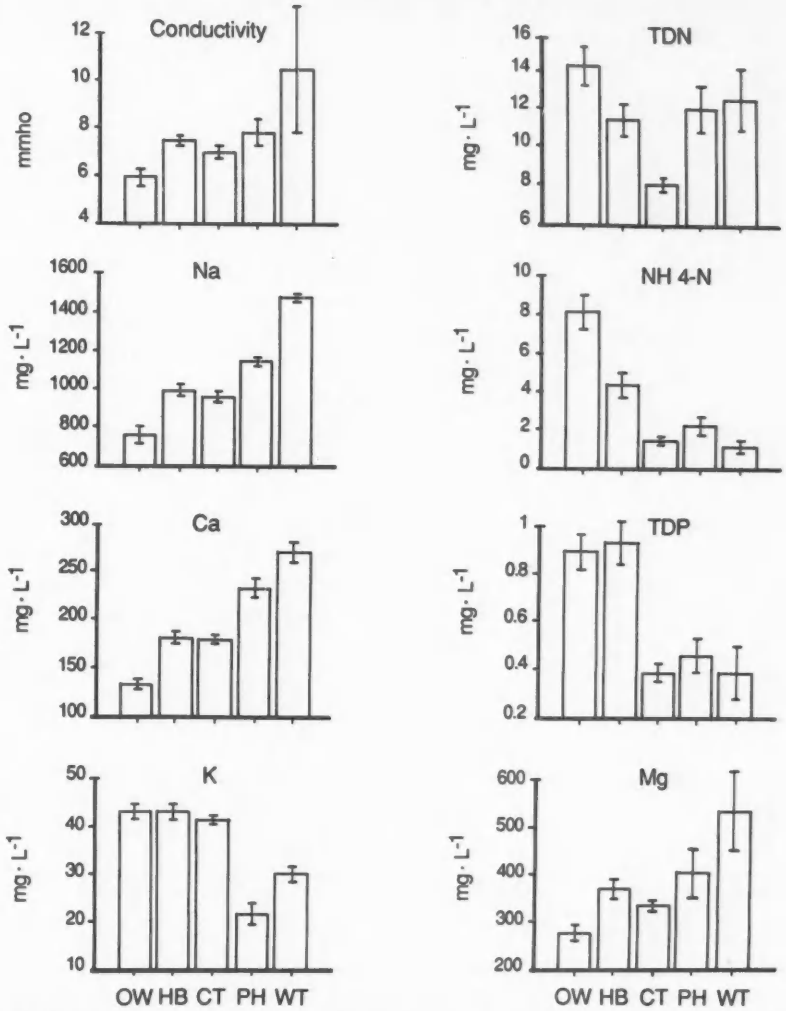


FIG. 2. Variation among vegetation types in concentrations of selected nutrients in interstitial water in 1980 at natural marsh water levels. Lines represent S.E. OW = open water, HB = hardstem bulrush, CT = cattail, PH = phragmites, and WT = whitetop.

permitting an ex post facto analysis of the effects of vegetation (Fig. 2). All constituents of interstitial water, except conductivity, showed large and significant differences (ANOVA) among vegetation (cover) types. The arrangement of bars in Fig. 2 reflects decreasing water depth from left to right — whitetop (WT) has fewest samples because many well sites were dry at 15 cm by mid- to late summer. Given that vegetation and station elevation tend to be related, the effects of these two factors are confounded in the statistical analyses.

In surface water, one-way ANOVA indicated significant differences for all constituents (except suspended C) among months. Most constituents had peak concentrations in June or later in summer. Seasonal changes in constituents in interstitial water were less pronounced and significant only for pH, conductivity, NH_4^+-N, TDN, and Mg^{2+}. As an example of variability among cell groups and months, the data for K^+ are graphed in Fig. 3.

Effect of Flooding

Because of the large number of samples taken, statistical comparisons by two-way ANOVA among the three years and three treatments almost always showed some significant differences, i.e. at least one pair of years or treatments was different for each constituent. Not all of these differences were due to the flooding. Logically, an effect of flooding should have appeared in the group 1 cells (1,2,4,5,6,8,9,10) in 1981 and continued in 1982. Group 2 cells (3 and 7) should not have shown a response to flooding until 1982, unless there was some seepage effect from adjoining group 1 cells in 1981. Group 3 cells (11 and 12), at normal water levels, should show only natural annual variations. Statistically, these patterns of annual change are a subset of the possible interactions in two-way ANOVA. Therefore, to assess the effects of flooding, I looked first for a significant interaction term and then at the pattern of the interactions.

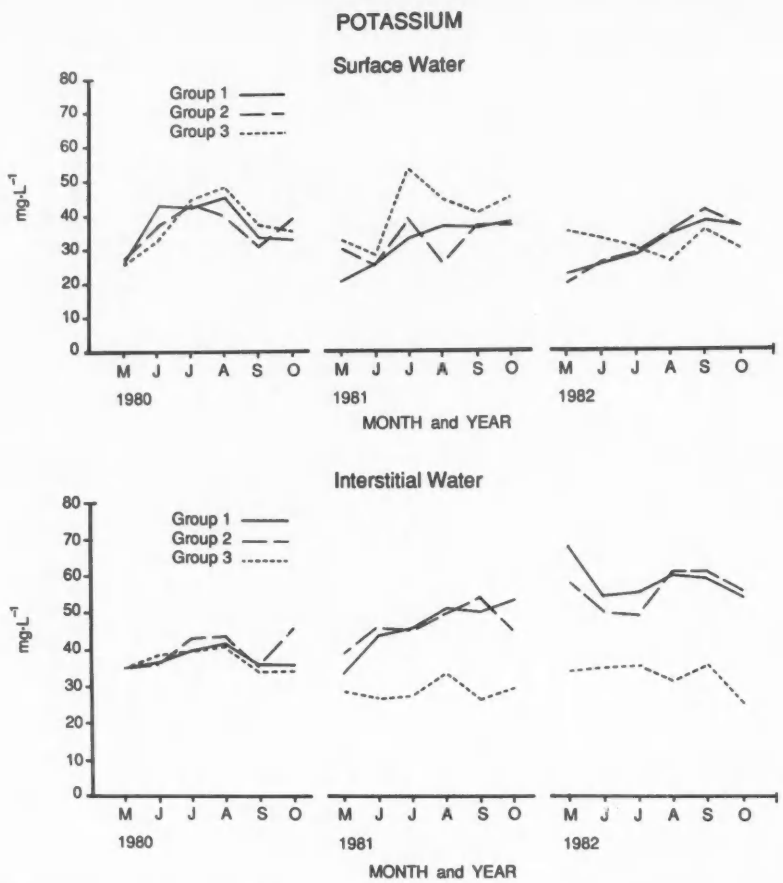


FIG. 3. Seasonal and annual variation in the concentrations of K^+, 1980–82. Monthly data points connected to facilitate comparisons.

Effects of flooding, by the above criteria, were observed for SRP, TDP, NH_4^+-N, and TDN in interstitial water (Fig. 4). Concentrations of all four of these constituents were very similar among the three cell groups in 1980. In 1981, the flooded cells (group 1) showed sharp increases in these forms of N and P whereas the unflooded groups 2 and 3 remained similar to 1980 prior to flooding. The group 2 cells, adjacent to the flooded cells, showed small (but sometimes statistically significant) increases in these constituents 1981, but when cells 3 and 7 were flooded in 1982, SRP, TDP, NH_4^+-N, and TDN approached levels similar to the group 1 cells in their first year of flooding, 1981. A second year of flooding in treatment 1 (1982) produced still higher levels of these forms of N and P.

In surface water, the dissolved forms of N and P did not show flooding effects, but there were treatment group and year effects (Fig. 5). For example, SRP concentrations were higher in the control cells (group 3) in every year, but they did not differ among years. However, TDN tended to increase from 1980 to 1982, and at least some of increases were significant, but there were no differences among cell groups.

N and P in the material suspended in surface waters showed very large changes both with time and as an effect of flooding (Fig. 6). In cell group 3, the cells in the undiked marsh, suspended N and P increased greatly in 1981 and 1982. Along with the higher concentrations was a large increase in variability, probably reflecting a patchy distribution of the suspended material, presumably mostly algae (S. M. Hosseini and A. G. van der Valk, pers. comm.). In contrast, the flooded cells, treatment 1 in 1981 and 1982, treatment 2 in 1982, showed sharp decreases in suspended N and P (Fig. 6).

Constituents other than the forms of N and P did not show responses to flooding. For example, Ca^{2+} and Mg^{2+} showed little evidence of consistent trends in either surface or interstitial water (Fig. 6). Indeed, Ca^{2+} in surface water was not significantly different among years in spite of very small standard errors. In interstitial water, variation in these constituents, although often statistically significant, and therefore probably real, did not seem to reflect any observed environmental differences.

Discussion

Nutrient Levels

In general, the concentrations of all constituents in the surface and interstitial water of these marsh units are very high

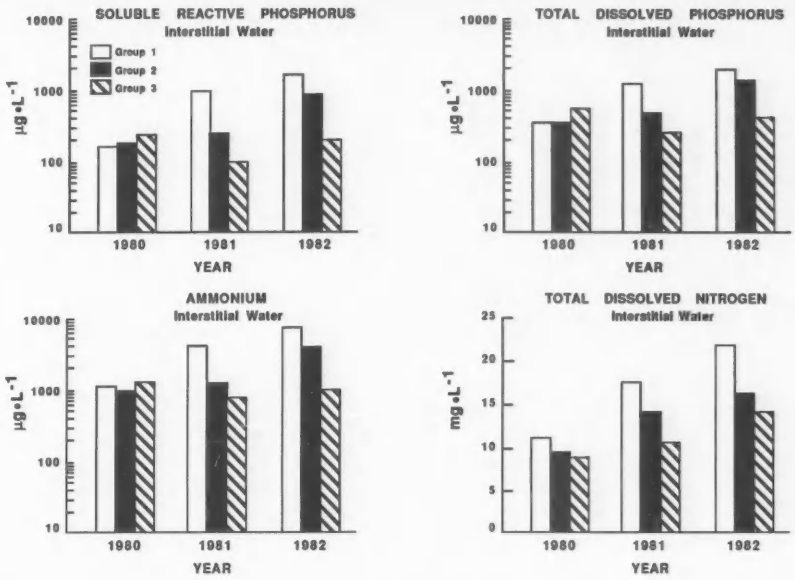


FIG. 4. Effects of flooding on mean annual concentrations of NH_4^+-N, TDN, SRP, and TDP in interstitial water, 1980–82. Group 1 cells (n = 8) flooded in 1981 and 1982; group 2 (n = 2) flooded in 1982; group 3 (n = 2) not flooded.

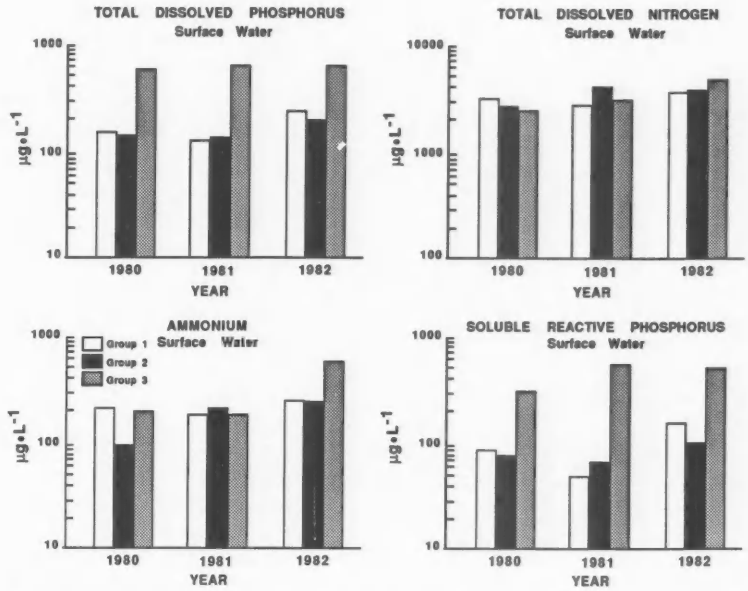


FIG. 5. Effects of flooding on mean annual concentrations of NH_4^+-N, TDN, SRP, and TDP in surface water, 1980–82. Group 1 cells (n = 8) flooded in 1981 and 1982; group 2 (n = 2) flooded in 1982; group 3 (n = 2) not flooded.

compared with most inland freshwater ecosystems (Wetzel 1975). The high level of conductivity (about 3000 $\mu mho \cdot cm^{-1}$ in surface water in the control cells during this study) is more typical of inland saline waters (Bierhuizen and Prepas 1985) and closed basins. Lake Manitoba and the Delta Marsh are remnants of glacial Lake Agassiz, which was fresh and well-drained (Cole 1975, p. 94). Present day Lake Manitoba is a flow-through system, but the high levels of salts in local ground water and the marsh reflect a closed basin during the postglacial period, about 8700–4000 yr B.P. (Teller and Last 1979).

Phosphorus and nitrogen are very abundant; total P averaged over 300 $\mu g \cdot L^{-1}$, higher than most values for freshwater wet-

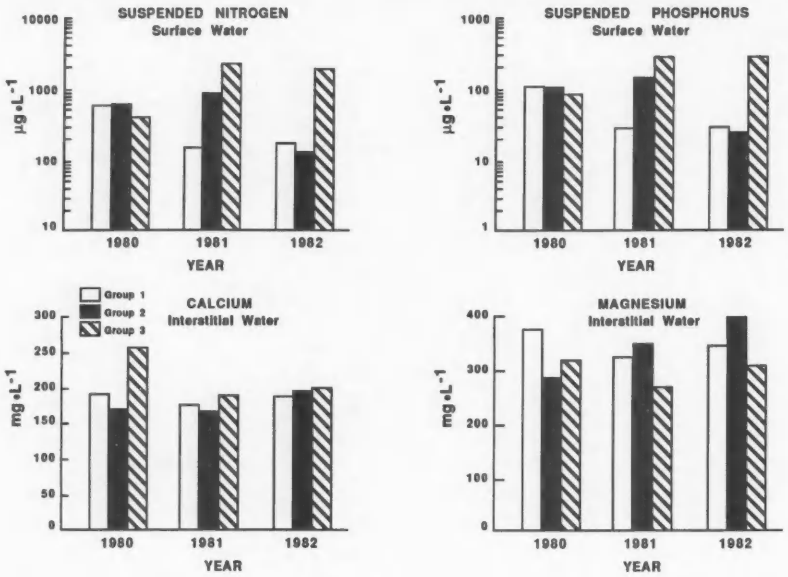


FIG. 6. Effects of flooding on mean annual concentrations of suspended N and P in surface water and Ca^{2+} and Mg^{2+} in interstitial water. Group 1 cells ($n = 8$) flooded in 1981 and 1982; group 2 ($n = 2$) flooded in 1982; group 3 ($n = 2$) not flooded.

lands reported by Kadlec (1979). Total organic N, approximated by the sum of suspended N plus TDN minus NH_4-N, averaged over 3.5 $mg \cdot L^{-1}$. In contrast, inorganic N, almost totally in NH_4^+-N form, averaged about 200 $\mu g \cdot L^{-1}$ and is at the low end of the ranges reported by Kadlec (1979). In contrast with freshwater lakes, saline lake algal standing crops are lower for given nutrient levels (Bierhuizen and Prepas 1985), making the interpretation of these high nutrient levels difficult. Further, Bierhuizen and Prepas (1985) suggested that algal response to nutrients depends on both ionic composition and the TN:TP ratio. The marshes studied herein differ from published studies of saline prairie lakes and ponds in being dominated by Na^+ and Cl^- rather than Ca^{2+} or Mg^{2+} and SO_4^{2-} or HCO_3^- (Barica 1975; Driver and Peden 1976; Bierhuizen and Prepas 1985). The low TN:TP ratio of about 10, low NH_4^+, and domination by Na^+ and Cl^- suggest the possibility of N limitation.

The vertical gradient in conductivity and most of the major ions, increasing with depth, was also found to be common in Great Salt Lake marshes (Kadlec 1982) but not in the Houghton Lake, Michigan, peatland (Kadlec 1976). In both cases of increases with depth, the ions are very abundant, certainly in excess of plant requirements. Consequently, the gradient is assumed to be the result of a set of physical processes operating between a relatively fresh surface water layer and a saline layer at some depth in the sediments. The data suggest that the steepest part of the gradient is in the upper 15 cm of the sediment, where conductivity increases 40–50% with depth, compared with about a 30% increase from 15 to 45 cm. In contrast, coastal salt marshes have high salt concentrations at the sediment surface and salinity decreases with depth (Howes et al. 1985). This difference in vertical salinity profile reflects the differing hydrologic conditions and source of salt in coastal marshes compared with the Delta and Salt Lake marshes.

The differences in concentration between surface water and interstitial water for the dissolved forms of N and P are very large. Even if one includes the suspended forms of N and P in the surface water, the amounts in interstitial water are still far greater. However, in contrast with the major ions, the amounts of NH_4^+-N, SRP, TDP, and K^+ decrease with depth within the sediments. The highest concentrations of NH_4^+-N, SRP, TDP, and K are therefore in the upper levels of the sediments. TDN is more evenly distributed in the sediments (Table 2), and there was not a significant difference among depths.

The departure of the vertical profiles of N, P, and K^+ from the profiles for conductivity and other major ions is probably due to the importance of N, P, and K^+ to plant growth. The profiles observed may have resulted from macrophyte uptake in 1980, but in 1981 and 1982 most of the macrophytes were dead (Murkin 1983). Decomposition in the upper sediment layers (e.g. Bowden 1984 for NH_4^+-N production) is always important, but death of roots and rhizomes after flooding probably increased N, P, and K release within the sediments (Carignan 1985). The gradient of redox potential may also contribute to the observed profiles in the sediments. Anaerobic conditions were common in the sediments — most interstitial water samples smelled strongly of H_2S. NO_3^- was always very low, even in surface water (1981 average only 2.4 $\mu g \cdot L^{-1}$), and probably has an ephemeral existence in this system. Hemond (1983) concluded that NO_3^- had only a transient existence in a bog system. In the anaerobic sediments, P should be in the dissolved form, but both NH_4^+ and PO_4^{2-} are adsorbed on fine organic or clay particles. As a consequence, the concentrations of both of these ions in interstitial water reflect exchange equilibria (Avnimelech et al. 1983) as well as total amounts. TDN was very abundant (≥ 10 $mg \cdot L^{-1}$) in interstitial water and did not display a strong vertical gradient. TDN must have been largely dissolved organic forms (DON), presumably decom-

position products. The high levels suggest an accumulation because of low rates of conversion of DON to other forms of N, perhaps because of anaerobic conditions.

Effect of Flooding

One of the effects of flooding was to increase the hydraulic head which forced water down through the bottom sediments, but more importantly out laterally through the dikes (Kadlec 1983 and unpubl.). The water pumped in was very similar in major ion composition to surface water in the cells, and therefore much more dilute (conductivity about 3500 μmho) than even the upper layers of interstitial water (about 7000 μmho at 15 cm). As the dilute water moved downward, interstitial water should have become more dilute. In fact, dilution in the interstitial water was very slight or absent (e.g. Ca^{2+} and Mg^{2+} in Fig. 6), strongly suggesting that most seepage was laterally through the dikes.

The largest changes from 1980 to 1981 and 1982 involved the forms of N and P in both surface and interstitial water. Apparently the control marshes (group 3, cells 11 and 12) had an enormous increase in algae in 1981 and 1982. S. M. Hosseini and A. G. van der Valk (pers. comm.) found that phytoplankton production averaged 39 μg $C \cdot L^{-1} \cdot h^{-1}$ in the control marshes in 1982 compared with 2.5 and 3.0 μg $C \cdot L^{-1} \cdot h^{-1}$ in the flooded group 1 and 2 marshes, respectively. This may have been the result of higher NH_4^+-N and SRP in the surface water, but similar levels of NH_4^+-N and SRP in the flooded marshes apparently produced floating filamentous algae instead. Suspended N, P, and C decreased in the flooded marshes even though higher concentrations were being pumped in (on the order of 100 μg suspended $P \cdot L^{-1}$, 1000 μg suspended $N \cdot L^{-1}$, and 8 mg suspended $C \cdot L^{-1}$, Kadlec, unpubl. data). The standing crop of floating filamentous algae in the flooded group 1 marshes in 1982 averaged 66 g ash-free dry $wt \cdot m^{-2}$ compared with 2.5 g ash-free dry $wt \cdot m^{-2}$ in the control marshes (S. M. Hosseini, pers. comm.). It is not clear why the flooded marshes, with deeper water and more wave action, produced filamentous algae and the shallow protected pools in the undiked marshes produced phytoplankton.

Increases in the nutrients NH_4^+-N, TDN, SRP, and TDP in interstitial water as a result of flooding suggest a net movement of nutrients to the sediments in the flooded cells. Several mechanisms might account for this shift. The flooded cells were more exposed to wind and wave action due to death of emergent vegetation (Murkin 1983), which might have altered the redox profile. Another possibility is that the death of the macrophyte vegetation, especially roots, and decomposition transferred nutrients directly to the sediment pore water (Carignan 1985). Avnimelech et al. (1983) also found higher NH_4^+-N pools in the sediment with increasing depth of the overlying water column. They attributed the increased NH_4^+-N to higher nutrient deposition and lower redox potential in deeper water.

My first prediction about the effects of flooding on nutrient concentrations turned out to have been overly simplistic. Both macrophyte (Murkin 1983) and phytoplankton (S. M. Hosseini, pers. comm.) standing crops decreased as a result of flooding. In surface water, neither SRP nor NH_4^+-N increased or decreased, relative to undiked control areas. Instead, SRP, TDP, NH_4^+-N, and TDN increased in interstitial water, where the availability of N and P for primary production, in the virtual absence of rooted macrophytes, is indirect at best. These observations do not preclude an increase in productivity following flooding, but do suggest that periphyton and filamentous algae are likely to be the basis for an increase if it does exist.

The second prediction, that major ion concentrations in interstitial water would decrease, was not supported by the data. There may have been some slight decrease, but even the large number of samples in this study was not sufficient to demonstrate a statistically significant decrease. In any event, it is clear that there was not a large seepage into the bottom sediments in spite of an hydraulic head of about 1 m. Seepage losses were substantial (Kadlec 1983), but must have been largely through the dikes.

Apparently changed physical conditions, such as water depth and wave action, and nutrient release due to death and decomposition of the macrophytes were responsible for the observed changes in nutrient concentrations due to flooding. However, physical factors, except for water depth, and internal processes were not monitored quantitatively, so the specific mechanisms remain to be confirmed by future studies.

Acknowledgements

This study was supported by the Delta Waterfowl and Wetlands Research Station and Ducks Unlimited Canada. I thank Bruce Batt, Patrick Caldwell, Henry Murkin, Jeff Nelson, and Arnold van der Valk for their review of this and earlier drafts of the manuscript. Chemical analyses were performed and supervised by John Baughen and Michael Stainton of the Freshwater Institute, Department of Fisheries and Oceans, Winnipeg, Manitoba.

References

AVNIMELECH, Y., M. YAMAMOTO, AND R. G. MENZEL. 1983. Evaluating the release of soluble components from sediment. J. Environ. Qual. 12: 86–91.

BARICA, J. 1975. Geochemistry and nutrient regime of saline eutrophic lakes in the Erickson–Elphinstone district of southwestern Manitoba. Fish. Mar. Serv. Res. Dev. Tech. Rep. 511: 82 p.

BATT, B. D. J., P. J. CALDWELL, C. B. DAVIS, J. A. KADLEC, R. M. KAMINSKI, H. R. MURKIN, AND A. G. VAN DER VALK. 1983. The Delta Waterfowl Research Station – Ducks Unlimited Canada marsh ecology research program, p. 19–23. *In* H. Boyd [ed.] First Western Hemisphere waterfowl and water bird symposium, Edmonton, Alberta, 25–28 May, 1982. Canadian Wildlife Service, Ottawa, Ont.

BIERHUIZEN, J. F. H., AND E. E. PREPAS. 1985. Relationship between nutrients, dominant ions, and phytoplankton standing crop in prairie saline lakes. Can. J. Fish. Aquat. Sci. 42: 1588–1594.

BOWDEN, W. B. 1984. A nitrogen-15 isotope dilution study of ammonium production and consumption in a marsh sediment. Limnol. Oceanogr. 29(5): 1009–1015.

CARIGNAN, R. 1985. Nutrient dynamics in a littoral sediment colonized by the submersed macrophyte *Myriophyllum spicatum*. Can. J. Fish. Aquat. Sci. 42: 1303–1311.

COLE, G. A. 1975. Textbook of limnology. Mosby, Saint Louis, MO. 283 p.

COOK, A. H., AND C. F. POWERS. 1958. Early biochemical changes in the soils and waters of artificially created marshes in New York. N.Y. Fish Game J. 5: 9–65.

DRIVER, E. A., AND D. G. PEDEN. 1976. The chemistry of surface water in prairie ponds. Hydrobiologia 53: 33–48.

HECKY, R. E., R. W. NEWBURY, R. A. BODALY, K. PATALAS, AND D. M. ROSENBURG. 1984. Environmental impact prediction and assessment: the Southern Indian Lake experience. Can. J. Fish. Aquat. Sci. 41: 720–732.

HEMOND, H. R. 1983. The nitrogen budget of Thoreau's Bog. Ecology 64: 99–109.

HOWES, B. L., J. W. H. DACEY, AND S. G. WAKEHAM. 1985. Effects of sampling technique on measurements of porewater constituents in salt marsh sediments. Limnol. Oceanogr. 30: 221–227.

HURLBERT, S. H. 1984. Pseudoreplication and the design of ecological field experiments. Ecol. Monogr. 54: 187–211.

KADLEC, J. A. 1962. The effect of a drawdown on the ecology of a waterfowl

impoundment. Ecology 43: 267–281.

1976. Dissolved nutrients in a peatland near Houghton Lake, Michigan, p. 25–91. *In* D. L. Tilton, R. H. Kadlec, and C. J. Richardson [ed.] Freshwater wetlands and sewage effluent disposal, Proc. of Symp., May 10–11, 1976. University of Michigan, Ann Arbor, MI.

1979. Nitrogen and phosphorus dynamics in inland freshwater wetlands, p. 17–41. *In* T. A. Bookhout [ed.] Waterfowl and wetlands — an integrated review. North Central Section, The Wildlife Society, Madison, WI.

1982. Mechanisms affecting salinity of Great Salt Lake marshes. Am. Midl. Nat. 107: 82–94.

1983. Water budgets for small diked marshes. Water Resour. Bull. 19: 223–229.

KU, W. C., F. A. DIGIANO, AND T. H. FENG. 1978. Factors affecting phosphate adsorption in lake sediments. Water Res. 12(12): 1069–1074.

MURKIN, H. R. 1983. Responses by aquatic macroinvertebrates to prolonged flooding of marsh habitat. Ph.D. thesis, Utah State University, Logan, UT. 112 p.

NEELY, R. W., AND C. B. DAVIS. 1985. Nitrogen and phosphorus fertilization of *Sparganium eurycarpum* Engelm. and *Typha glauca* Godr. stands. I. Emergent plant production. Aquat. Bot. 22: 347–361.

NIE, N. H., C. H. HULL, J. G. JENKINS, K. STEINBRENNER, AND D. H. BENT. 1975. Statistical package for the social sciences. McGraw-Hill, New York, NY.

STAINTON, M. P., M. J. CAPEL, AND F. A. J. ARMSTRONG. 1977. The chemical analysis of freshwater. 2nd ed. Can. Fish. Mar. Serv. Misc. Spec. Publ. 25: 180 p.

TELLER, J. T., AND W. M. LAST. 1979. Post-glacial sedimentation and history in Lake Manitoba. Report to Dep. of Mines, Nat. Resour. and Environ., Prov. of Manitoba. University of Manitoba, Winnipeg, Man. 185 p.

VAN DER VALK, A. G., AND C. B. DAVIS. 1978. The role of seed banks in the vegetation dynamics of prairie glacial marshes. Ecology 59: 322–335.

WELLER, M. W. 1979. Wetland habitats, p. 210–234. *In* P. E. Greeson, J. R. Clark, and J. E. Clark [ed.] Wetland functions and values: the state of our understanding. American Water Resources Association, Minneapolis, MN.

WETZEL, R. G. 1975. Limnology. Saunders, Philadelphia, PA. 743 p.

Input–Output Nutrient Budgets for Small Diked Marshes[1]

John A. Kadlec

Department of Fisheries and Wildlife, Utah State University, Logan, UT 84322-5200, USA

Kadlec, J. A. 1986. Input–output nutrient budgets for small diked marshes. Can. J. Fish. Aquat. Sci. 43: 2009–2016.

Input–output nutrient budgets were constructed for 8 and 10 diked contiguous 4- to 6-ha marshes in 1981 and 1982, respectively. Because the marshes studied were created by diking within a larger marsh, the water budgets were dominated by water pumped in to replace roughly equal evapotranspiration and seepage losses. Chloride budgets could not be used to confirm water budgets, apparently because of a transfer to the sediments by a freezing-out process in winter and a subsequent diffusion back into the surface water during the ice-free season. With replicated ponds and accurate water budgets, good agreement was found among replicates for annual budgets for the major ions and total N. However, the budgets for total P were highly variable, reflecting the biological activity of P. Atmospheric inputs of N and P were large components of budgets for these nutrients. Year to year increases in surface water concentrations of major ions, expected on the basis of evaporative water loss, did not occur, apparently because of freezing-out of these ions in winter.

Nous avons établi respectivement en 1981 et 1982 les bilans d'entrée–sortie des nutriments pour 8 et 10 marais endigués contigus de 4 à 6 ha. Étant donné que ces marais ont été créés par endiguement d'un marais plus grand, les bilans hydrologiques ont été dominés par l'eau amenée par pompage pour remplacer des pertes à peu près égales causées par l'évapotranspiration et l'infiltration. Il n'a pas été possible d'utiliser des bilans des chlorures pour confirmer les bilans hydrologiques, du fait apparemment que ces ions étaient transférés dans les sédiments par un processus de gel hivernal puis rediffusés dans l'eau de surface pendant la saison sans glace. Avec l'emploi de marais semblables et des bilans hydrologiques précis, nous avons trouvé une bonne concordance dans les bilans annuels des principaux ions et du N total. Toutefois, les bilans du P total étaient très variables, ce qui reflète l'activité biologique de P. Les apports atmosphériques de N et P constituaient des éléments importants des bilans de ces nutriments. Les augmentations d'une année à l'autre des concentrations des principaux ions dans l'eau de surface, qui étaient prévues du fait des pertes d'eau par évaporation, ne se sont pas produites, ce qui est apparemment lié à l'effet du gel hivernal.

Received November 26, 1985
Accepted June 24, 1986
(J8590)

Reçu le 26 novembre 1985
Accepté le 24 juin 1986

Input-output nutrient budgets provide the basic framework for understanding internal nutrient cycling in aquatic ecosystems. In lake systems this is often expressed by the concepts of nutrient loading or input to retention time ratios (Wetzel 1983). In streams, the concept of nutrient "spiralling" (Webster and Patten 1979) serves a similar function. Because nutrient cycling in marshes and wetlands has been little studied, a firm conceptual base has not been established, with borrowing both from limnology (e.g. Kadlec 1979) and from stream ecology (e.g. Howard-Williams 1985). Input–output budgets serve to summarize the net function of the ecosystem, but yield little or no quantitative information on the mechanisms by which the net results are achieved. Conversely, detailed quantitative studies of mechanisms are unlikely to provide much insight into net function, if for no other reason than the impracticality of adequately studying all processes simultaneously as they vary over time and space. Nutrient cycles in freshwater marsh ecosystems are particularly difficult to assess internally because of heterogeneity of macrophyte spatial distribution and the lack of a unifying physical organizing factor such as water depth or unidirectional water flow.

Input–output nutrient budgets also are important for understanding potential nutrient removal functions of wetlands. The nutrient removal functions of wetlands have received increasing attention in recent years (Kadlec and Kadlec 1979; Richardson 1985). In spite of this, the Office of Technology Assessment (1984) review was only able to locate seven studies that they believed to be reasonably reliable for input–output measures of N and P. Several problems make such studies difficult: (1) a good water budget is a necessary prerequisite (LaBaugh and Winter 1984); (2) all inputs and outputs of water must be monitored frequently for chemical composition (Scheider et al. 1979), requiring large numbers of chemical analyses; and (3) the systems are inherently highly variable (Kadlec 1986), complicating sampling and inferential statistics. Good water budgets for wetlands are rare (Carter et al. 1979) partly because of technological problems. Recently, Winter (1981) and LaBaugh and Winter (1984) have pointed out that the methods for estimating many of the terms in a water budget equation involve biases or uncertainties that imply wide confidence intervals for the estimates. Because nutrient budgets involve the multiplication of water volumes by chemical constituent concentrations, both with high levels of uncertainty, the estimates of the products must have very broad confidence intervals, although such are rarely reported. Similar problems have been recognized in lake studies (Cross and Rigler 1983).

[1]Paper No. 18 of the Marsh Ecology Research Program, Delta Waterfowl and Wetlands Research Station – Ducks Unlimited Canada.

The purpose of this study was to combine the water budget and nutrient data to construct input–output nutrient budgets for eight diked marshes in 1981 and 10 marshes in 1982. In the long term, we want to evaluate the effects of water level manipulation on nutrient cycling, but the immediate goal of this study was to estimate the precision with which we can calculate overall input–output budgets as a necessary first step in examining nutrient cycles. Secondary goals included (1) examining changes in ion concentrations in relation to a known hydrology and (2) estimating the relative magnitude of various sources and sinks of nutrient ions.

Methods

Field

Ten contiguous 4- to 6-ha marsh compartments (cells) were constructed in 1979 by diking within the large Delta Marsh in south-central Manitoba. Descriptions of the Delta Marsh and the diked cells are available in Kadlec (1983), as are a description of the methods used to calculate water budgets. Briefly, the principal water input was by pumping via meters. Rainfall on the marsh surfaces was estimated as the product of the amount of rain measured at two on-site standard rain gauges and measured marsh areas. Surface water inflow was considered negligible because it could occur only from dike surfaces. Changes in stored water volumes were estimated from water level records (staff gauges) and stage-volume tables based on 10-cm-interval contour lines from detailed topographic mapping of the site. As explained in Kadlec (1983), seepage was considered constant over time, although cells differed, and thus seepage loss was simply the product of the daily rate and the number of days in each month. Seepage rates were mostly estimated by difference, but seepage meters and calculations based on hydraulic conductivity were used to cross check the results. Evapotranspiration (ET) was considered constant across cells, but daily and monthly rates varied substantially. Several techniques were used to approximate ET, including standard evaporation pans, evaporation pans partly submersed in the marsh, a modified Penman equation (Eagleson 1970), and calculation as the difference in the water mass balance equation when seepage could be independently estimated.

Monthly water budgets for the growing season, roughly May through October, were constructed by summing all rainfall and pumped inputs for each month, calculating the net change in storage between the beginning and end of the month, and estimating by difference total water loss for the month. A water budget for the growing season of each of 1981 and 1982 was calculated by summing the monthly budgets.

In surface water and precipitation, the chemical constituents monitored included Cl^-, SO_4^{2-}, Na^+, K^+, Ca^{2+}, Mg^{2+}, soluble reactive P (SRP), total dissolved P (TDP), NH_4^+-N, NO_3^--N, total dissolved N (TDN), suspended P, suspended N, and suspended C. Analytical techniques follow Stainton et al. (1977), and surface and interstitial water sampling techniques are detailed in Kadlec (1986).

Atmospheric nutrient inputs, both precipitation and dryfall, were sampled by collecting rainfall in polyethylene bottles with funnel collectors at each of the two weather stations at the study area. After each precipitation event the water was transferred to standard sample bottles and stored at 4°C. Collections for each month were composited by station, giving two samples each month, and analyzed as described for surface water. Water being pumped into the cells was sampled weekly by grab samples at the pump intakes.

Calculations

Monthly water budgets were calculated in terms of water volumes (cubic metres). Pumped water was metered in cubic metres but other terms in the budget were measured or estimated in units of millimetres per month and were multiplied by the surface area of each cell to obtain volumes. For purposes of calculating monthly changes in storage of nutrients, the total volume of water in each cell was calculated for the beginning of each month from water level data and stage-volume tables.

Nutrient budgets in terms of mass were calculated for each month by multiplying mean chemical concentrations by the appropriate water volumes. The procedure was straightforward for water being pumped into the cells and atmospheric inputs. Seepage losses across the sediment–water interface were assumed to carry the same concentration as surface water. Monthly changes in storage were the difference between estimates of the amount stored on the first day of successive months. Amount stored was calculated as the product of water volume in the cell and the nutrient concentration estimated for that date and cell. Because nutrient concentrations changed seasonally, it was necessary to assume linear changes between monthly mean concentrations and interpolate the concentrations for the first of each month. This assumption introduced some error (probably about ±3%, Scheider et al. 1979), but trials of other procedures revealed even greater problems.

For each constituent, mass balances were calculated by summing inputs and subtracting increases in storage and outputs. The difference is a result of either errors in the estimates of the terms in the budget or the net effect of internal processes such as sediment–water interactions, or both. Some estimate of inherent error was possible by comparing the replicates. Because all cells were not of equal size, comparisons were standardized by converting all mass balance budgets to a mass per hectare basis.

Results

Water Budgets

Water levels were held constant during the growing season by pumping water into the marshes to replace seepage and evapotranspiration losses. Consequently, these terms dominate the water budgets (Table 1). Seepage was substantial because water levels inside the dikes were about 1 m above the surrounding marsh. For cells with a long (approximately 700 m) dike with 1-m hydraulic head difference (cells 2, 4, 6, and 8 in 1981 plus 1 and 10 in both years), seepage equaled or exceeded ET. When a cell was flanked by flooded cells (cells 5 and 9 in 1981, cells 2–9 in 1982), reducing potential seepage through the dike, seepage was usually much less than ET.

Because the inputs to each cell of pumped water and rainfall were measured directly, as was change in storage, the total volume of water leaving each cell was known, by difference, quite accurately (Table 1). The variations among cells in these quantities represent real differences, except for measurement error of rain gauges and the meters on the pumps. Changes in storage, probably most subject to measurement error, were largest in spring of 1981 when pumping was necessary to replace overwinter seepage losses.

TABLE 1. Seasonal water budgets for the flooded cells of the Marsh Ecology Research Program, Delta Waterfowl and Wetlands Research Station, Manitoba. Volumes in m^3 converted to $mm \cdot d^{-1}$ for purposes of comparisons. Means and S.E. for cells similar in length of perimeter through which seepage could occur.

Cell	(1) Pumped (metered)	(2) Precip. (two gauges)	(3) ΔV[a]	(1 + 2 −3) Total out[b] (calculated)	(4) Seepage	(5) ET[c]
			April 18 − October 29, 1981			
1	5.34	2.32	1.22	6.44	4.08	2.36
2	4.24	2.32	1.22	5.34	2.98	2.36
4	5.69	2.32	1.48	6.53	3.60	2.93
5	2.92	2.32	1.18	4.06	1.82	2.24
6	5.35	2.32	1.51	6.15	3.46	2.69
8	4.62	2.32	1.33	5.61	3.17	2.44
9	3.77	2.32	1.30	4.79	2.40	2.39
10	5.25	2.32	1.29	6.53	3.93	2.60
$\bar{x}$[d]	5.08	2.32	1.34	6.10	3.54	2.56
SE	0.22		0.05	0.21	0.17	0.09
$\bar{x}$[e]	3.35	2.32	1.25	4.43	2.11	2.32
SE	0.43		0.06	0.37	0.29	0.08
			May 1 − September 28, 1982			
1	5.80	2.16	1.05	6.91	3.48	3.43
2	3.03	2.16	0.66	4.53	1.10	3.43
3	3.57	2.16	0.92	4.81	1.38	3.43
4	2.99	2.16	0.37	4.78	1.35	3.43
5	2.63	2.16	0.27	4.52	1.09	3.43
6	2.84	2.16	0.33	4.67	1.24	3.43
7	2.65	2.16	0.47	4.34	0.91	3.43
8	3.14	2.16	0.20	5.10	1.67	3.43
9	2.66	2.16	0.00	4.82	1.39	3.43
10	4.87	2.16	0.28	6.75	3.34	3.43
$\bar{x}$[f]	5.43	2.16	0.67	6.83	3.40	3.43
SE	0.47		0.39	0.08	0.04	
$\bar{x}$[g]	2.94	2.16	0.40	4.70	1.27	3.43
SE	0.11		0.10	0.08	0.08	

[a]Change in storage from stage-volume tables (see text).
[b]Total out is the total water loss due to seepage and evapotranspiration.
[c]Evapotranspiration.
[d]Cells 1,2,4,5,8,10; each flanked on one side by an unflooded marsh.
[e]Cells 5 and 9; both with flooded marshed on both sides.
[f]Cells 1 and 10; flanked on one side by unflooded marsh.
[g]Cells 2−9; flanked on both sides by flooded marsh.

Water losses from each cell were the sum of seepage and ET. In 1981, these were approximated from a number of different measurements (see Kadlec 1983) and then adjusted to meet as closely as possible the criteria of a constant seepage rate for each cell across time and approximately equal monthly ET rates across cells. Because of heterogeneous sediments and different lengths of dike through which seepage could occur, the cells were expected to differ in rates of seepage loss. Because flooding inundated and killed most of the vegetation (Murkin 1983), variation among cells in ET was expected to be minimal.

In 1982, cells 1, 5, 9, and 10 were unchanged from 1981 in terms of local hydrology, and as a first approximation their seepage rates were assumed to be the same as in 1981. These rates were used to calculate a mean seasonal (May 1 − September 28) average daily ET (Table 1) rate and then all seepage rates recalculated. That procedure resulted in cells 2−9, all with flooded cells adjacent on both sides and therefore limited lateral seepage, having low and similar seepage rates (Table 1). Cells 1 and 10 had higher seepage rates, very similar to the average rates for cells 1,2,4,6,8, and 10 in 1981, which also had one long dike through which seepage could occur. The 1982 seasonal ET rate of 3.43 $mm \cdot d^{-1}$ derived by this approach was similar to the rate of 3.25 $mm \cdot d^{-1}$ of evaporation from two Class A pans sunk in the marsh (marsh pans), a rate of 3.51 $mm \cdot d^{-1}$ derived from 0.7 times (Carter et al. 1979) the average evaporation from two standard Class A pan installations, and a rate of 3.37 $mm \cdot d^{-1}$ based on a modified Penman approach (Eagleson, 1970, p. 221). This rate was higher for 1982 than 1981 because the 1982 season did not include late April and October whose low ET rates reduced the 1981 seasonal average.

Average monthly ET rates derived from the calculations outlined above, assuming constant seepage, were lower but followed Class A pan evaporation rates quite closely (Fig. 1). Notable dips in ET rates based on water budgets occurred in August 1981 and July 1982, in both cases months of higher

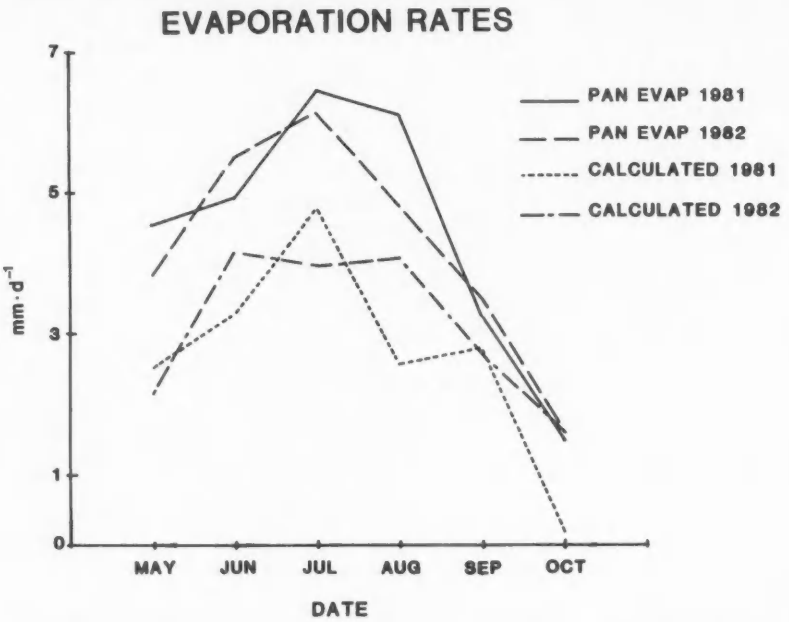


FIG. 1. Comparison of seasonal course of marsh evapotranspiration estimated from Class A pans and water budgets. Monthly data points connected to show similarities among years and differences between methods.

TABLE 2. Mass balance budgets for the major ions and total N and P, Marsh Ecology Research Program, Delta Marsh, Manitoba, 1981 and 1982. Means (SE) for eight cells in 1981 and 10 cells in 1982.

	Pumped	Rainfall and dryfall[a]	Change in storage	Seepage	Net[b]
Cl ($t \cdot ha^{-1}$)					
1981	7.32(0.64)	0.01	3.62(0.17)	4.55(0.39)	−0.83(0.19)
1982	3.96(0.45)	0.02	3.30(0.22)	1.78(0.09)	−1.08(0.17)
SO_4 ($t \cdot ha^{-1}$)					
1981	3.27(0.41)	0.02	1.16(0.13)	1.60(0.18)	0.53(0.16)
1982	1.76(0.27)	0.02	1.27(0.14)	0.64(0.14)	−0.13(0.12)
Na ($t \cdot ha^{-1}$)					
1981	4.71(0.40)	0.01	2.19(0.10)	2.86(0.25)	−0.37(0.11)
1982	2.69(0.12)	0.01	2.44(0.17)	1.20(0.21)	−0.94(0.17)
K ($kg \cdot ha^{-1}$)					
1981	276(20)	4	206(13)	195(15)	−121(11)
1982	162(15)	4	161(10)	77(13)	−72(10)
Ca ($kg \cdot ha^{-1}$)					
1981	614(73)	16	211(13)	413(38)	+10(37)
1982	362(53)	8	213(28)	162(28)	−4(38)
Mg ($t \cdot ha^{-1}$)					
1981	1.55(0.16)	0.01	0.92(0.06)	0.96(0.09)	−0.32(0.05)
1982	0.87(0.12)	0.01	0.61(0.06)	0.39(0.07)	−0.13(0.05)
Total N ($kg \cdot ha^{-1}$)					
1981	28.22(2.37)	12.08	26.67(1.36)	17.86(1.78)	−4.23(1.15)
1982	21.65(2.69)	6.59	15.10(1.69)	9.93(1.87)	+3.21(1.55)
Total P ($kg \cdot ha^{-1}$)					
1981	1.93(0.18)	1.13	1.07(0.24)	1.07(0.24)	+0.92(0.41)
1982	1.58(0.49)	1.05	1.05(0.28)	0.68(0.18)	+0.94(0.63)

[a]Based on the same data for all cells, thus no SE possible.
[b]A negative indicates an internal source and a positive an internal sink.

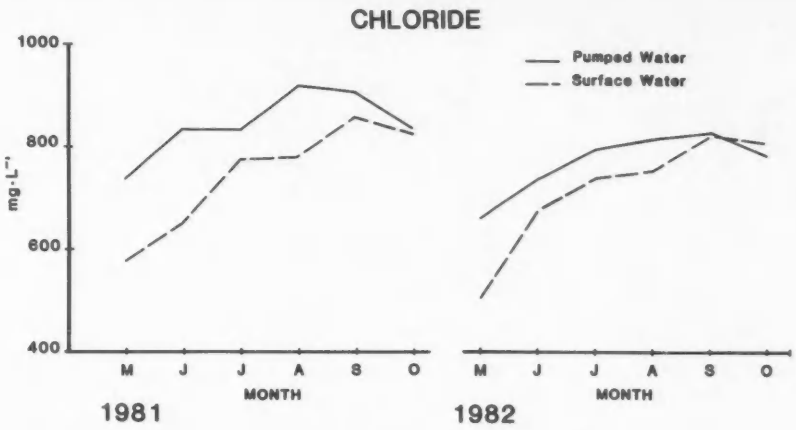


FIG. 2. Seasonal changes in Cl concentrations in surface water in the cells and in the water pumped into the cells. Monthly data points connected to facilitate comparison.

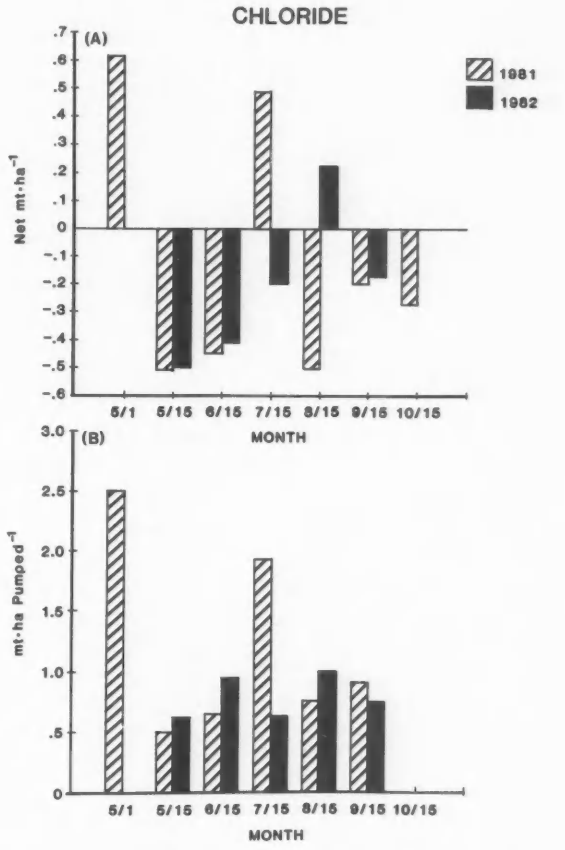


FIG. 3. (A) Net and (B) pumped terms in monthly Cl budgets.

rainfall. Apparently, rainfall depresses marsh ET more than Class A pan evaporation.

In summary, the input and change in storage parts of the water budgets (Table 1) were measured directly and are therefore quite reliable. Seepage and ET estimates were derived, and although I believe they are reasonable, the rates could be changed 0.2 $mm \cdot d^{-1}$ and still be reasonable. According to LaBaugh and Winter (1984), this is approximately the error to be expected in ET and precipitation measurements. Greater variation would be inconsistent with other estimates of ET

TABLE 3. Seasonal changes in surface water nutrient concentrations due to summer evaporation and winter freeze-out plus dilution, 1981–82, in cells 1,2,4,5,6,8,9, and 10, Marsh Ecology Research Program, Delta Waterfowl and Wetlands Research Station, Manitoba.

	1981				1982			
	May (n = 40)		October (n = 35)		May (n = 42)		September (n = 44)	
	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
Na ($mg \cdot L^{-1}$)	366	8	514	7	337	5	565	6
K ($mg \cdot L^{-1}$)	21	1	39	<1	23	<1	39	<1
Ca ($mg \cdot L^{-1}$)	61	1	68	1	53	1	66	1
Mg ($mg \cdot L^{-1}$)	116	3	193	4	115	2	167	3
Cl ($mg \cdot L^{-1}$)	571	12	825	10	499	7	811	15
SO_4 ($mg \cdot L^{-1}$)	205	7	278	11	170	6	305	12
TDN ($\mu g \cdot L^{-1}$)	1276	90	3960	82	2741	95	3985	75
TDP ($\mu g \cdot L^{-1}$)	57	10	174	19	156	22	195	36

(Class A pans and marsh pans), the assumptions outlined earlier for seepage and ET, or non-negativity constraints on seepage and ET. Seepage varied with length of dike through which seepage could occur, but approximately equaled ET in cells having seepage through one long (approximately 700 m) dike.

Nutrient Budgets

Chloride

Chloride budgets are sometimes recommended as a check on water budgets (Kadlec and Kadlec 1979). In this study, I anticipated using the Cl budget to refine seepage estimates, because all other terms in the Cl mass balance (Table 2) were much more accurately determined. However, as the 1981 monthly mass balances were calculated, it was obvious that some systematic error was involved, and rather than correct the 1981 water budgets, the 1982 water and Cl mass balances were calculated as described. In the 1982 data, at least one source of difficulty was obvious: increases in the Cl storage in the cells were greater than the total Cl added in many months, especially in spring. This is a physical impossibility and therefore the data, calculations, and assumptions involved in mass balances were carefully reexamined. Reducing seepage losses to zero, a highly unlikely possibility, did not help. Atmospheric inputs of Cl were neglible. Although there certainly was enough variability in both the storage and pumped terms to account for the error, chance variations in those measurements should have resulted in a random assortment of over- and underestimates, rather than the consistent patterns observed. Finally, R. H. Kadlec (University of Michigan, Ann Arbor, MI, pers. comm.) suggested that Cl and similar ions might be forced out the water column and into the sediments by winter freezing, which at this latitude penetrates into the sediments, and then be released back into the surface water after the spring thaw. This mechanism has been observed in tundra ponds by Prentki et al. (1980) whose graphs of changes in Cl concentration in surface water during the ice-free season are remarkably similar to those observed in this study (Fig. 2). Because freezing out and subsequent diffusion back into the surface water is a mechanism not accounted for in the Cl budgets calculated for Table 2, it is not possible to use the Cl budget to verify the water budget. Therefore, the water budgets as presented in Table 1 remain the best estimates possible with the available data.

Although the mass balance of Cl gave a negative "net" term in most months (Fig. 3A), consistent with the postulated diffusion from the sediments, in three months the net term of the budget was positive. In May and July 1981, the positive balances correlated with high pumped Cl inputs (Fig. 3B). In 1981 there was a strong statistical relationship ($r^2 = 0.96$, $t = 9.98$, $p < 0.001$) between the net term and the pumped input. In essence, this means that the change in storage did not increase proportionally to the increased pumped inputs. Conversely, the low net balance in August 1981 corresponded to a month of high precipitation, with low Cl input, that was not reflected in low or negative change in storage for the month. In general, it appears that the change in storage, as calculated herein, is damped and does not reflect monthly changes very well. This is probably an artifact of monthly sampling mixed with both continuous and pulse inputs, outputs, and change in storage (Scheider et al. 1979). However, when averaged over the ice-free season, as in Table 2, I believe the results are reliable.

The monthly pattern of changes in Cl concentration (Fig. 2) suggested that the freeze-out phenomenon might be responsible for returning ion concentrations in surface water to similar levels each spring. The cells flooded in fall 1980 allowed an examination of that possibility. Evaporative concentration led to increases in concentration during the summer of 1981, as Fig. 2 illustrates for Cl. Overwinter decreases could be partly due to dilution by early spring runoff and pumping, but concentration decreased much more (nearly 40%) than the added volume (average 10%) of dilute water could explain. The generality of this phenomenon is indicated by spring–fall–spring comparisons of all the dissolved ion concentrations (Table 3). All ion concentrations increased from May to October 1981 as a result of evaporative concentration and other processes affected by flooding (e.g. decomposition, anaerobiasis). All the major ions decreased from October 1981 (just prior to freeze-up) to concentrations in May 1982 (shortly after spring break-up) approximately equal to those in May 1981. Overwinter decreases in total N and P were less pronounced and May 1982 concentrations were much higher than May 1981.

Other major ions

The budgets for Cl^- and SO_4^{2-} and the major cations (Table 2) all show the reduced pumped inputs in 1982 because of the change in the hydrology in many of the cells. In 1982, cells 2,4,6, and 8 had less dike through which seepage would occur, thus reducing average seepage losses.

Most of the net balances for these major ions were negative, presumably reflecting the freezing-out phenomena discussed above. Ca^{2+} and SO_4^{2-} depart from that pattern, showing no significant difference from 0 ($p > 0.05$, t-test), except in 1981 for SO_4^{2-}. Among the major ions, K^+ and SO_4^{2-} are most active biologically, and therefore, changes are less likely to be accounted for solely by transport processes. As expected, pumping accounted for most of the input of all the major ions.

Phosphorus and nitrogen

Total N variability was similar to the major ions, but total P budgets were somewhat more variable among the marshes, as

indicated by higher SE (Table 2). Inputs by rainfall and dryfall were high relative to pumped inputs, especially for total P. The very large SE for the net terms for total P means that those terms do not differ from zero in the statistical sense. Included in those SE is variability known to be caused by differences among cells in seepage rates. Marshes 5 and 9 in 1981 and 2–9 in 1982 had much lower seepage rates and hence lower amounts of water pumped. Considering those groups of cells separately did not change the conclusion that the net terms do not differ from zero.

Discussion

Water Budgets

Among adjacent, hydrologically similar cells, total variability in water budgets is best expressed in the "Total out" column in Table 1. For each group of cells in each year, coefficients of variation (CV) ranged from 1.6% for eight similar cells in 1982 to 11.7% for two similar cells in 1981. This low variability is a combination of real variation in water loss plus measurement error, primarily in changes in storage. Precipitation was considered a constant and thus does not contribute to the variability. According to LaBaugh and Winter (1984), even standard rain gauges are subject to 10–20% error. Our use of the mean of two gauges should have reduced that error, but an overall error of 0.1–0.2 mm·d^{-1} (Table 1) might be possible. This would not affect the precision (CV) of the estimates of total water losses, but could affect their accuracy; they might be as much as 0.2 mm·d^{-1} too high or low. That is still a very small percentage bias in the total water loss. I conclude that the estimates of total water loss are very reliable.

Partitioning of the total water loss between ET and seepage is less reliable. The concordance of four independent calculations of ET (range 3.25–3.51 mm·d^{-1} in 1982) supports the value calculated from the water budget data (3.43 mm·d^{-1}). However, given Winter's (1981) appraisal of these techniques, I see no a priori reason to assume that any one of these values is more valid than the others. An error of 0.2 mm·d^{-1} appears quite possible.

Given potential errors, or biases, of up to 0.2 mm·d^{-1} in both precipitation and ET estimates, the seepage rates in Table 1 might easily be up to 0.4 mm·d^{-1} too high or too low. For cells with high seepage rates, that is only about a 12% error. But for cells with low rates, such as cells 2–9 in 1982, that is over a 30% error in the average and 44% for cell 7. Fortunately, the volumes of water involved are relatively small, so the impacts on nutrient budgets are less serious.

Nutrient Budgets

Coefficients of variation in the terms of the budgets for total P, even under relatively controlled conditions with good water budgets, ranged from 25 to 100%. However, for the major ions such as Ca^{2+}, Na^+, Mg^{2+}, K^+, Cl^-, and SO_4^{2-}, and also for total N, good agreement was found among the replicates on an annual basis. This indicates that random error, in a statistical sense, was not excessive. However, simple input–output budgets may not balance even for conservative ions such as Na^+ and Cl^- because of biases or processes not accounted for in the measurements (e.g. freeze-out). Consequently, it should come as no surprise that conclusions about biologically more active, and therefore more variable, constituents such as P are tenuous at best.

The budgets for both Cl^- and Na^+ strongly suggest that winter freezing is an important phenomenon in nutrient relationships in shallow water in northern regions. Several studies (e.g. Barica 1977; Canfield et al. 1983; Daborn and Clifford 1974) have shown increased ion concentrations in the water column below the ice, and some limited sampling at the Delta Marsh showed conductivities of water below the ice in winter to be similar to those characteristic of interstitial water in summer. Except for Prentki et al. (1980), the effects of freezing the entire water column and upper sediment layers have not been reported. Summerfield and Rieley (1973) investigated alternately freezing and thawing peat cores in the laboratory and found a release of K. R. H. Kadlec (pers. comm.) has done laboratory studies of freezing into the sediments which suggest that the effects on various ions may differ depending on rates as well as depth of freezing. All ions are affected to some degree, suggesting that the freezing-out phenomenon may be of importance in the cycles of critical nutrients.

The importance of atmospheric inputs in the budgets of total N and total P was unexpected in view of the large volumes of water pumped into the diked marshes. Our measures of atmospheric inputs combined dry fall (dust) with rainfall inputs, suggesting that the estimates of total inputs might be biased upwards because of improper weighting of the dust component. Comparison of our data with ranges given by Wetzel (1983, p. 224 and 280) suggests that this might be true for N, but only marginally or not at all for P. Relatively high values for K^+, Ca^{2+}, and Mg^{2+} might also reflect dust inputs. Both 1981 and 1982 were drought years on the Canadian prairies and dust storms were not uncommon. Dry fall (dust) inputs of nutrients seem to be regionally variable and are often substantial (Swank 1984).

Internal Mechanisms of Nutrient Change

The contents of the residual compartment, for purposes of these budgets, were the sediments and biota. Several internal functions of the this compartment are clear from the data. First, the freezing-out phenomenon apparently resulted in the sediments being a sourse for Na^+, Cl^-, K^+, and Mg^{2+} for at least the early part of the growing season as well as being a sink for those, and possibly all, ions in winter.

Most other studies of marsh nutrient budgets (e.g. Cole and Fisher 1979; Phillips 1977) concentrate on the fraction of nutrient inputs retained by the marsh. In this study, the only pathways for nutrient loss were seepage and freeze-out, both of which move nutrients into the sediments. The budgets indicated that seepage loss was less than inputs for all ions in both years, which would lead to the expectation of continued increases in concentration. This was true oniy for TDP (Table 3). For the major ions, freeze-out apparently prevented build-up from year to year, as discussed earlier. TDN showed some additional carryover through the winter of 1981–82, but subsequently increased less in the summer of 1982 than in 1981. Given the multiple pathways by which the forms of N are converted, etc., this complex behavior may not be unusual.

Acknowledgements

This study was supported by the Delta Waterfowl and Wetlands Research Station and Ducks Unlimited Canada. I thank Bruce Batt, Patrick Caldwell, Henry Murkin, Jeff Nelson, and Arnold van der Valk for their review of the manuscript. Chemical analyses were

performed and supervised by John Baughen and Michael Stainton of the Freshwater Institue, Department of Fisheries and Oceans, Winnipeg, Manitoba.

References

BARICA, J. 1977. Effect of freeze-up on minor ion and nutrient content of a prairie winterkill lake. J. Fish. Res. Board Can. 34: 2210–2215.

CANFIELD, D. E., R. W. BACHMANN, AND M. V. HOYER. 1983. Freeze-out of salts in hard-water lakes. Limnol. Oceanogr. 28: 970–977.

CARTER, V., M. S. BEDINGER, R. P. NOVITSKI, AND W. O. WILEN. 1979. Water resources and wetlands, p. 344–376. *In* P. E. Greeson, J. R. Clark, and J. E. Clark [ed.] Wetland functions and values: the state of our understanding. American Water Resources Association, Minneapolis, MN.

COLE, J. J., AND S. G. FISHER. 1979. Nutrient budgets of a temporary pond ecosystem. Hydrobiologia 63(32): 213–222.

CROSS, P. M., AND F. H. RIGLER. 1983. Phosphorus and iron retention in sediments measured by mass budget calculations and directly. Can. J. Fish. Aquat. Sci. 40: 1589–1597.

DABORN, G. R., AND H. F. CLIFFORD. 1974. Physical and chemical features of an aestival pond in western Canada. Hydrobiologia 44(1): 43–59.

EAGLESON, P. S. 1970. Dynamic hydrology. McGraw-Hill, New York, NY. 462 p.

HOWARD-WILLIAMS, C. 1985. Cycling and retension of nitrogen and phosphorus in wetlands: a theoretical and applied perspective. Freshwater Biol. 15: 391–431.

KADLEC, J. A. 1979. Nitrogen and phosphorus dynamics in inland freshwater wetlands, p. 17–41. *In* T. A. Bookhout [ed.] Waterfowl and wetlands — an integrated review. North Central Section, The Wildlife Society, Madison, WI.

1983. Water budgets for small diked marshes. Water Resour. Bull. 19: 223–229.

1986. Effects of flooding on dissolved and suspended nutrients in small diked marshes. Can. J. Fish. Aquat. Sci. 43: 1999–2008.

KADLEC, R. H., AND J. A. KADLEC. 1979. Wetlands and water quality, p. 436–456. *In* P. E. Greeson, J. R. Clark, and J. E. Clark [ed.] Wetland functions and values: the state of our understanding. American Water Resources Association, Minneapolis, MN.

LABAUGH, J. W., AND T. C. WINTER. 1984. The impact of uncertainties in hydrologic measurement on phosphorus budgets and empirical models for two Colorado reservoirs. Limnol. Oceanogr. 29: 322–339.

MURKIN, H. R. 1983. Responses by aquatic macroinvertebrates to prolonged flooding of marsh habitat. Ph.D. thesis, Utah State University, Logan, UT. 112 p.

OFFICE OF TECHNOLOGY ASSESSMENT. 1984. Wetlands: their use and regulation. U.S. Congress, OTA-0-206, Washington, DC.

PHILLIPS, G. L. 1977. The mineral nutrient levels in three Norfolk broads differing in trophic status, and an annual mineral content budget for one of them. J. Ecol. 65: 447–474.

PRENTKI, R. T., M. C. MILLER, R. J. BARSDATE, V. ALEXANDER, J. KELLEY, AND P. COYNE. 1980. Chemistry, p. 76–178. *In* J. E. Hobby [ed.] Limnology of tundra ponds. Dowden, Hutchinson and Ross, Stroudsburg, PA.

RICHARDSON, C. J. 1985. Mechanisms controlling phosphous retention capacity in freshwater wetlands. Science (Wash., DC) 228: 1424–1427.

SCHEIDER, W. A., J. J. MOSS, AND P. J. DILLON. 1979. Measurement and uses of hydraulic and nutrient budgets, p. 77–83. *In* Lake restoration. U.S. EPA 440/5-79-001, Washington, DC.

STAINTON, M. P., M. J. CAPEL, AND F. A. J. ARMSTRONG. 1977. The chemical analysis of freshwater. 2nd ed. Can. Fish. Mar. Serv. Misc. Spec. Publ. 25: 180 p.

SUMMERFIELD, R. J., AND J. O. RIELEY. 1973. Substrate freezing and thawing as a factor in the mineral nutrient status of mine ecosystems. Plant Soil 38: 557–566.

SWANK, W. T. 1984. Atmospheric contributions to forest nutrient cycling. Water Resour. Bull. 20: 313–321.

WEBSTER, J. R., AND B. C. PATTEN. 1979. Effects of watershed perturbation on stream potassium and calcium dynamics. Ecol. Monogr. 49: 51–72.

WETZEL, R. G. 1983. Limnology. 2nd ed. Saunders, Philadelphia, PA.

WINTER, T. C. 1981. Uncertainties in estimating the water balance of lakes. Water Resour. Bull. 17: 82–115.

Investigation sur la toxicodynamique du cyanure et sur sa biotransformation chez la truite arc-en-ciel (*Salmo gairdneri*)

Pierre Raymond, Gérard Leduc et Jack A. Kornblatt

Département de Biologie, Université Concordia, Montréal (Québec) H3G 1M8

Raymond P., G. Leduc et J. A. Kornblatt. 1986. Investigation sur la toxicodynamique du cyanure et sur sa biotransformation chez la truite arc-en-ciel (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 43: 2017–2024.

L'exposition de truites arc-en-ciel (*Salmo gairdneri*) à trois concentrations de cyanure, 0,01, 0,02 et 0,03 mg·L^{-1} de HCN pour des périodes de 20 j a démontré qu'il y a biotransformation du HCN en thiocyanate qui s'accumule rapidement dans le plasma sanguin et que cette concentration augmente avec la durée d'exposition. Bien que le cyanure soit transformé en thiocyanate, il en demeure toutefois une quantité résiduelle suffisante pour causer une réduction de 60–80 % de la cytochrome oxydase et ceci, dès les 24 premières h d'exposition au cyanure. Ce niveau d'inhibition est demeuré stable durant les 20 j de l'essai et on a noté qu'il était le même aux trois concentrations étudiées. Cette observation permet de prédire la concentration effective du HCN libre dans le poisson. Les mesures du glycogène hépatique mettent en lumière l'effet Pasteur chez la truite arc-en-ciel soumise au cyanure. Durant l'exposition, le glycogène hépatique fut activement utilisé, particulièrement à 0,03 mg·L^{-1} de HCN. Par contre, à 0,01 et 0,02 mg·L^{-1} de HCN, les niveaux de glycogène sont graduellement revenus à la normale après 7–10 j d'exposition.

The exposure of rainbow trout (*Salmo gairdneri*) to 0.01, 0.02 and 0.03 mg HCN·L^{-1} for 20 d demonstrated the biotransformation of HCN into thiocyanate which accumulated in the blood plasma which increased the duration of exposure to cyanide. Despite its transformation into thiocyanate, there is still a sufficient quantity of cyanide to inhibit cytochrome oxidase by 60–80% within the first 24 h of exposure. This level of inhibition remained stable during the 20 d of the tests and was the same at the three concentrations tested. This observation permits the prediction of effective concentration of free HCN inside the fish. The measurements of liver glycogen levels illustrate the Pasteur effect in the rainbow trout subjected to cyanide. During cyanide exposure, liver glycogen was rapidly used, particularly at 0.03 mg HCN·L^{-1}, whereas at 0.01 and 0.02 mg HCN·L^{-1} the glycogen levels gradually came back to normal after 7–10 d of exposure.

Reçu le 7 mars 1985
Accepté le 20 juin 1986
(J8141)

Received March 7, 1985
Accepted June 20, 1986

Le but de cette étude est d'éclaircir le mode d'action du cyanure (HCN) chez la truite arc-en-ciel (*Salmo gairdneri*) lors d'expositions prolongées à des concentrations sous-létales du toxique. À cette fin les paramètres suivants furent mesurés : l'activité de la cytochrome oxydase du foie, la bioaccumulation du thiocyanate dans le plasma sanguin et le glycogène hépatique.

En toxicologie aquatique, le cyanure a été l'objet d'innombrables études de toxicité aiguë et chronique sur le poisson (voir les revues bibliographiques de Doudoroff 1976, 1980; Leduc *et al.* 1982; Leduc 1984) mais peu d'attention a été donnée aux mécanismes d'intoxication. On attribue généralement la toxicité du cyanure à l'inhibition de la chaîne respiratoire des mitochondries qui conduit à l'épuisement des réserves d'ATP. D'autre part la glycolyse (effet Pasteur) peut pallier d'une façon limitée à la déficience énergétique durant l'intoxication (Vennesland *et al.* 1981; Solomonson 1981). Ces réponses sont bien connues chez les mammifères mais tel n'est pas le cas chez le poisson dont on ne connait à peu près rien de la toxicodynamique du cyanure.

À la suite de nombreuses études de la toxicité chronique du cyanure sur le poisson, il se dégage le schéma suivant : aux concentrations sous-létales variant entre 0,005 et 0,10 mg·L^{-1} de HCN, le cyanure peut affecter les capacités de reproduction, la croissance, la nage et plusieurs autres fonctions physiologiques vitales (Leduc 1984). Il est toutefois intrigant qu'il y ait eu à plusieurs reprises soit une stimulation soit une accélération du taux de croissance au cours d'expositions prolongées de poissons au cyanure (Leduc 1984).

De plus, si on expose des fretins de truites arc-en-ciel à une concentration sous-létale de cyanure, ces truites deviennent initialement plus sensibles au cyanure mais regagnent par la suite leur résistance (Dixon et Sprague 1981).

Ces réponses d'adaptation au cyanure ont été initialement attribuées soit à une augmentation d'activité de cytochrome oxydase (Leduc 1984) soit à la détoxification du cyanure qui est effectuée par la rhodanase, transformant le cyanure en thiocyanate ($^{-}$SCN) (Dixon et Sprague 1981). Cette fonction enzymatique bien connue (Way 1981; Westley 1981) a aussi été identifiée chez le poisson (Sido et Koj 1972; Schievelbein *et al.* 1969) et l'on peut s'attendre à une accumulation de $^{-}$SCN dans le plasma de poissons exposés à des concentrations sous-létales de cyanure (HCN).

Nous avons donc posé les questions suivantes :

1) Est-ce que l'activité de cytochrome oxydase dans le foie de poissons exposés au cyanure varie avec la durée de l'exposition?

2) Est-ce que le thiocyanate s'accumule dans le poisson exposé au cyanure?

3) Est-ce que les variations de niveaux de glycogène hépatique pourraient expliquer une adaptation au cyanure, tel que le suggèrent Kovacs et Leduc (1982)?

Matériel et méthodes

Pour qu'on puisse répondre aux questions posées plus haut, des truites arc-en-ciel ont été exposées pendant des périodes de 20 j à des concentrations sous-létales de cyanure. Ces poissons ont été échantillonés à divers intervalles de temps pour mesurer la cytochrome oxydase du foie, le glycogène hépatique et l'accumulation de $^-$SCN dans le plasma sanguin.

On a utilisé les truites arc-en-ciel achetées à la Pisciculture des Landes, à Saint-Félix de Kingsay (Québec). À leur arrivée au laboratoire (Université Concordia) les poissons furent transférés et gardés dans des bacs de réserve moulés de forme ovale, en fibre de verre, d'une capacité de 240 L et alimentés en eau courante (12°C ± 1°C). L'eau du laboratoire, qui provient de l'aqueduc de Montréal, est déchlorée, réchauffée ou réfrigérée au besoin et distribuée dans des tuyaux de plastique (C.P.V.).

Les poissons ont été nourris tous les deux jours au taux d'environ 1,8 % de leur poids de moulée à truite (Purina) n° 4P ou 6P selon la grosseur des poissons. Les poissons ont été gardés dans ces conditions pendant un minimum de deux semaines avant le début des essais, période où la mortalité n'a jamais excédé 0,5 %. La date des essais, la taille et le nombre des poissons sont donnés ci-dessous.

Essai et date	Longueur (cm) $\bar{X}$ (± DS)	Poids (g) $\bar{X}$ (± DS)	N poissons PAR BAC	N mortalités dans les essais, avant/durant (chez le groupe TÉMOIN)
1. oct. 81	24,9 (± 0,66)	163,2 (± 6,4)	27	3 2
2. févr. 82	26,7 (±0,29)	168,5 (± 0,7)	27	0 0
3. mai 82	15,8 (± 0,89)	32,3 (± 4,9)	32	0 0

Dans ces essais les truites ont été exposées pendant une période de 20 j, à 12°C, à des concentrations sous-létales de cyanure de 0,01, 0,02 et 0,03 mg·L^{-1} de HCN. Un groupe témoin était gardé sous les même conditions mais sans cyanure.

Tous les essais ont été faits dans des bacs semblables à ceux utilisés lors de l'arrivée des poissons au laboratoire. Avant le début des essais, les poissons acclimatés étaient distribués au hasard dans quatre bacs et gardés durant une semaine pendant laquelle ils étaient nourris quotidiennement soit aux taux de 1,1 % de la biomasse pour les poissons d'environ 165 g, soit de taux de 1,9 % pour ceux de 32 g (Leitritz et Lewis 1976). Ces taux furent maintenus durant toute la période expérimentale. Le débit d'eau dans les bacs était de 4 L·min^{-1}, permettant un remplacement de 99 % en 5 h (Sprague 1973). Les solutions de cyanure (KCN) ont été appliquées à débit constant par bouteille de Mariotte (Leduc 1966a). Les concentrations étaient vérifiées au moins deux fois au cours des essais par la méthode de Lambert *et al.* (1975); les fluctuations n'ont pas dépassé 10 % des valeurs prévues.

Les autres paramètres chimiques mesurés aux cours des essais furent l'oxygène dissous qui est demeuré au-dessus de 80 % de saturation, le pH qui s'est maintenu à une moyenne de 7,87, et l'alcalinité et la dureté de l'eau qui se situaient autour de 83 et 127 mg·L^{-1} de $CaCO_3$. L'illumination par tubes fluorescents était de 60–90 lux à la surface de l'eau et la photopériode contrôlée était de 12 h.

Protocole expérimental

Durant le premier essai, des truites d'environ 163 g ont été échantillonnées aux jours 0, 3, 6, 11 et 20 après le début de l'application du cyanure. Pour les deuxième et troisième essais on a utilisé des truites de 168 g et de 32 g échantillonnées aux jours 0, 1, 5, 10, 15 et 20. Le nombre de truites échantillonnées aux jours précités était de cinq pour les essais 1 et 2 et de six pour le troisième.

Au moment de l'échantillonnage, le poisson était rapidement tué d'un coup sur la tête puis mesuré et pesé. Le sang était immédiatement prélevé dans des tubes à centrifugation héparinisés après qu'on eut sectionné le pédoncule caudal. Les tubes étaient centrifugés à 3 000 rpm pendant 15 min puis le plasma était transféré dans des tubes Eppendorf et mis sur la glace en vue de la détermination du thiocyanate. Pour les petits poissons (32 g), le sang de deux ou trois individus était combiné pour obtenir un volume suffisant. On prélèvait ensuite le foie de chaque poisson et, après l'avoir pesé, on le congèlait rapidement dans l'azote liquide et on l'entreposait à −20°C en vue de mesurer l'activité de cytochrome oxydase et de glycogène.

Analyses biochimiques

Thiocyanate du plasma

Le thiocyanate du plasma a été mesuré moins de 24 h après le prélèvement, sauf pour les échantillons du jour 0 qui étaient analysés en même temps que ceux du jour un. Ce laps de temps est acceptable selon Ballantyne (1977).

Le plasma est déprotéinisé par l'addition de 9 volumes d'acide trichloroacétique 10 %, laissé à reposer 10 min à la température ambiante puis centrifugé à 3 000 rpm pendant 15 min. On détermine immédiatement le $^-$SCN par la méthode de Lambert *et al.* (1975) modifiée comme suit: on mélange 1 mL de surnageant avec 1 mL de tampon phosphate 1 *M* à pH 7,5, puis on ajoute 0,05 mL de N-chlorosuccinimide et 0,05 mL du réactif pyridine–acide barbiturique. On laisse incuber 12 min à 25°C puis on prend la densité optique à 575 nm. Tous les échantillon ont été mesurés deux fois.

L'activité de la cytochrome oxydase hépatique a été mesurée 48 h après l'échantillonnage selon le protocole suivant: le foie était broyé dans un mortier rempli d'azote liquide et réduit en une fine poudre qui était ensuite suspendue dans un tampon phosphate 0,02 *M* à raison de 1 g par 5 mL. On a homogénéisé la suspension gardée sur glace avec un microhomogénéisateur Sorvall à une vitesse de 21 500 rpm pendant 3 min en prenant soin de la réfrigérer toutes les 30 s. Les cellules demeurées intactes ont été séparées par centrifugation à 2 000 g et à froid. Le surnageant a été prélevé et dilué au besoin selon Smith (1955). La solution de réactif de cytochrome c (extrait de cœur de cheval, type III 95 %, Sigma Chemical) a été préparée à raison de 5 mg/mL de tampon phosphate (Yonetani 1966) et conservée à −20°C dans des cuvettes de 7 mL. Au moment d'une analyse, la quantité requise de solution était dégelée, on y ajoutait quelques grains de $Na_2S_2O_4$ solide et on y laissait barboter de l'azote durant 5 min pour assurer une réduction complète. La solution était ensuite purifiée sur colonne Sephadex (Yonetani 1966). Pour prévenir la formation de H_2O_2, oxydant du cytochrome c, la solution était à nouveau barbotée à l'azote pendant 5 min, à l'hydrogène pendant une heure à l'azote de nouveau pendant 5 min. La solution tamponnée de cytochrome c était alors diluée à 90 μM dans un tampon phosphate 0,01 *M* à pH 7,0 (Smith 1955). Toutes ces

TABLEAU 1. Composantes de la variance de la cytochrome oxydase hépatique chez les truites arc-en-ciel exposées au cyanure pendant 20 j dans les essais 2 (168,5 g) et 3 (32,2 g).

Facteur	Composante de variance	% de la variance totale	
		Essai 2	Essai 3
a	Durée de l'exposition	3,6	3
b	Concentration de cyanure	61,6	62
ab	Interaction	4,1	6
c(*ab*)	Poisson individuel	26,7	16
w	Erreur interne	3,8	12

opérations ont été exécutées en chambre froide et les réactifs gardés sur de la glace.

L'activité de la cytochrome oxydase a été mesurée deux fois par l'oxydation enzymatique du cytochrome c observée toutes les deux secondes à 550 nm sur un spectrophotomètre enregistreur PYE Unicam SP B-100, permettant de calculer l'activité comme une constante de cinétique de premier ordre (Smith 1955). La teneur totale de protéines de la suspension était mesurée le jour suivant par la méthode de Lowry (Cooper 1977).

Glycogène du foie

Les échantillons de foie conservés au congélateur pour les mesures de glycogène ont été analysés à la fin des essais de 20 j. Les foies étaient broyés en une fine poudre dans l'azote liquide puis digérés dans une solution de KOH à 30%. Le glycogène était précipité à l'alcool 95% selon la technique de Good *et al.* (1933) et mesuré par colorimétrie (Montgomery 1957).

Résultats

Thiocyanate sérique

La biotransformation du cyanure en thiocyanate ($^-$SCN) et son accumulation dans le plasma sanguin de truites arc-en-ciel exposées à différentes concentrations de HCN furent mesurées durant trois essais de 20 j (fig. 1). Les graphiques de la figure 1 montrent que le niveau de base de $^-$SCN chez les témoins a varié quelque peu d'un essai à l'autre mais pas de façon significative. Chez les truites exposées au cyanure, le $^-$SCN sérique exhibait une accumulation dès l'exposition au cyanure, qui s'accentuait avec la durée d'exposition.

Dans les essais 1 et 2 exécutés avec de grosses truites, l'accumulation de $^-$SCN a progressé de façon plus ou moins uniforme durant les 20 j d'exposition avec évidemment beaucoup plus de variation dans l'essai 1 que dans l'essai 2. À la fin des essais, il n'y avait pas de relation dose–réponse significative entre les niveaux de $^-$SCN dans le plasma et les niveaux d'exposition au cyanure. Par contre, dans l'ensemble, ces niveaux étaient plus élevés ($P < 0,05$) que ceux des témoins par un facteur de 300 à 700%.

Durant l'essai 3, à cause de la petite taille des poissons, les échantillons de plasma ont dû être combinés de sorte qu'il n'y a pas de données individuelles et d'analyse statistique possible. Dans cet essai nous avons observé une augmentation continuelle de $^-$SCN jusqu'aux jours 10 ou 15, suivie d'un déclin au jour 20. À la fin de l'essai, les niveaux de $^-$SCN à 0,01, 0,02 et 0,03 mg·L^{-1} de HCN étaient 202, 67 et 390% plus élevés que chez les témoins. Ces niveaux semblent moins élevés que dans les deux essais précédents.

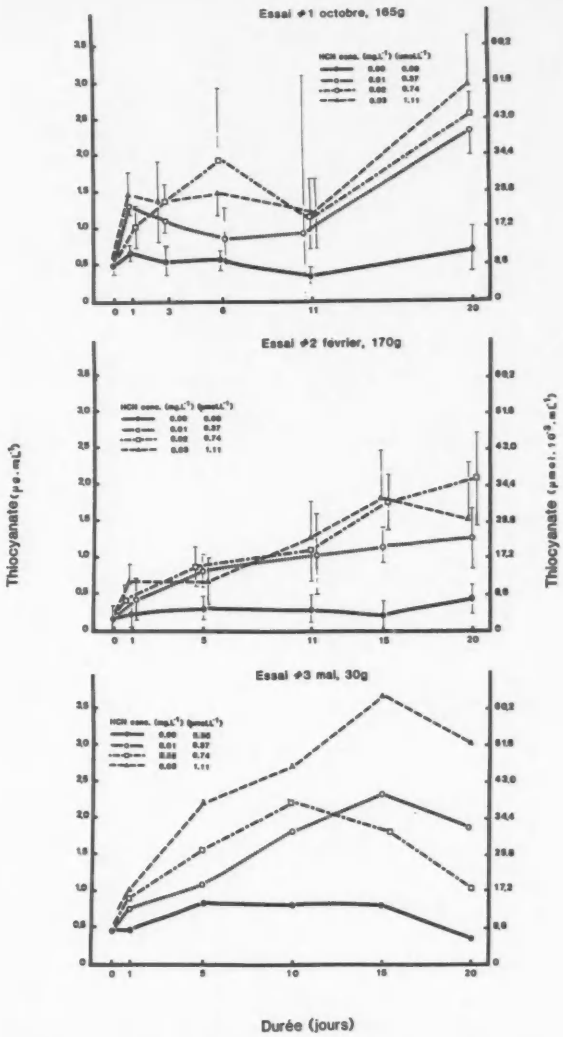


FIG. 1. Niveaux moyens de thiocyanate sérique et limites de confiance (95%) dans des truites arc-en-ciel de différents poids exposées pendant 20 j à différentes concentrations de cyanure, à 13°C, durant les essais 1, 2 et 3.

Puisque les essais ont été effectués à différents temps de l'année et que par surcroît les données de l'essai 3 ont été combinées, il n'est pas possible d'établir de relation quantitative entre le taux d'accumulation de $^-$SCN et le poids des poissons. Tout au plus pouvons-nous suggérer une accumulation plus rapide chez les spécimens plus petits au début, suivie d'une baisse appréciable.

Cytochrome oxydase

L'activité de la cytochrome oxydase hépatique, mesurée à divers intervalles durant des périodes de 20 j d'exposition au cyanure dans les essais 2 et 3 (fig. 2), indique une inhibition marquée dès les premières heures d'exposition.

Dans l'essai 2 nous avons observé chez les témoins une grande variation d'activité d'un échantillon à l'autre, probable-

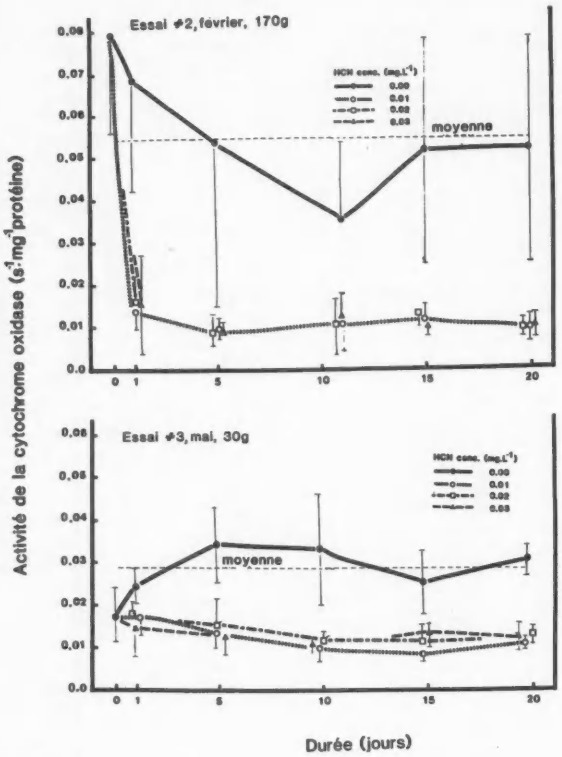


FIG. 2. Activité moyenne de cytochrome oxydase hépatique et limites de confiance (95 %) de truites arc-en-ciel de différents poids exposées pendant 20 j à différentes concentrations de cyanure, à 13°C, durant les essais 2 et 3.

ment due aux différences individuelles. Par contre les valeurs des poissons exposés au cyanure sont très uniformes, montrant une inhibition immédiate et constante. Pour faciliter l'interprétation des résultats, on a calculé la moyenne arithmétique des données du groupe témoin des jours 0 à 20, et on a obtenu une valeur de $0{,}0537 \cdot s^{-1} \cdot mg^{-1}$ de protéine. Par contre les poissons exposés au cyanure n'ont pas démontré de relation dose–réponse, les niveaux d'activité étant à peu près les mêmes aux trois concentrations. De plus, l'activité est demeurée semblable d'un jour à l'autre. Pour ces raisons on a aussi calculé le moyenne arithmétique des données de tous les poissons exposés au cyanure des jours 1 à 20, et on a obtenu une valeur de $0{,}0104 \cdot s^{-1} \cdot mg^{-1}$ de protéine, soit une réduction d'activité d'environ 80 % par rapport aux témoins. Cette différence est hautement significative ($P < 0{,}001$) telle que vérifiée par analyse de variance (three-way ANOVA, Lindman 1974) (tableau 1). Cette analyse, exécutée pour identifier des sources de variation révèle que malgré des limites de confiance très larges (95 %), la cytochrome oxydase était réduite de 80 % en moins de 24 h aux trois concentrations de cyanure.

L'analyse des résultats de l'essai 3 a été faite selon la même approche que pour l'essai 2. La moyenne arithmétique des poissons témoins se situait à $0{,}0272 \cdot s^{-1} \cdot mg^{-1}$ de protéine, soit à peu près la moitié de la valeur observée chez les truites témoins de 168 g. Comme dans le cas précédent nous n'avons pas décelé de relation dose–réponse.

Si on calcule la moyenne pour tous les poissons exposés au cyanure, on obtient une valeur de $0{,}0113 \cdot s^{-1} \cdot mg^{-1}$ de protéine, soit une différence d'environ 58 % par rapport aux témoins. Cette différence est significative ($P < 0{,}001$). Comme dans l'essai précédent, l'analyse de variance (voir tableau 1) a révélé que l'effet de réduction d'activité était significativement relié au cyanure.

Il est intéressant de noter que pour les deux groupes de tailles de truites et à différents temps de l'année, l'activité minimum de cytochrome oxydase à n'importe quelle des trois concentrations de cyanure était essentiellement la même, soit 0,0104 et $0{,}0107 \cdot s^{-1} \cdot mg^{-1}$ de protéine dans les essais 2 et 3. Il semble donc qu'en dépit des différences entre les valeurs témoins (essais 2 et 3) l'inhibition maximale ait été atteinte dans les deux cas.

Glycogène hépatique

Le glycogène hépatique des truites témoins et expérimentales a été mesuré sur une période de 20 j au cours de l'essai 1; les données sont présentées au tableau 2. Nous avons observé d'importantes fluctuations dans tous les groupes, et pour tenter de mieux démarquer l'effet du cyanure, nous avons eu recours à la normalisation des données qui sont illustrées en graphique dans la figure 3.

Les niveaux de glycogène des poissons empoisonnés ont eu tendance à baisser au début de l'exposition au cyanure puis à augmenter ou à se stabiliser selon les concentrations de HCN. À 0,01 $mg \cdot L^{-1}$ il n'y a pas eu de différences significatives par rapport aux témoins, sauf pour une baisse au jour 6 suivie d'une remontée. À 0,02 et 0,03 $mg \cdot L^{-1}$ de HCN, le glycogène est tombé significativement sous la valeur des témoins au jour 3, a continué sa chute aux jours 6 et 11, puis, pour 0,03 $mg \cdot L^{-1}$ de HCN, s'est stabilisé à une valeur inférieure ($P < 0{,}01$) aux témoins jusqu'au jour 20. Par contre, à 0,02 $mg \cdot L^{-1}$ de HCN le glycogène a effectué une remontée au jour 11 pour se situer, au jour 20, à une valeur semblable à celle des témoins.

Les changements temporels notés se sont révélés significatifs ($P < 0{,}001$) de même que ceux reliés aux concentrations de cyanure ($P < 0{,}01$), tel que confirmé par une analyse de variance (three-way ANOVA, Lindman 1974).

Discussion

Toxicocinétique

Nos résultats ont montré une accumulation rapide de ^{-}SCN en moins de 24 h d'exposition à toutes les concentrations de cyanure étudiées. Ce phénomène concorde bien avec les études antérieures de toxicité chronique du cyanure sur des mannifères incluant l'homme (Barrett *et al.* 1978; Bourdoux *et al.* 1978; Chandra *et al.* 1980). Chez les truites témoins (fig. 1), un niveau de base de thiocyanate est commun parce que le cyanure est très répandu dans la nature et provient de nombreux générateurs, comme les cyanoglycosides (Solomonson 1981). La majeure partie du thiocyanate dans un organisme provient de la détoxification du CN^{-} (Wolff 1964) en passant par l'activité de la rhodanase (thiosulfate: cyanure sulfotransférase, EC 2.8.1.1.) Westley 1981; Way 1981), enzyme présente chez le poisson (Schievelbein *et al.* 1969; Sido et Koj 1972).

Il est bon de rappeler ici que même si nous n'avons pas observé de relation dose–réponse entre les niveaux de HCN dans l'eau et ceux de ^{-}SCN dans le plasma des truites exposées, au jour 20 le ^{-}SCN dans ces mêmes truites (156–170 g)

TABLEAU 2. Moyennes de glycogène hépatique, écart type et limites de confiance à 95 % chez les truites arc-en-ciel (165 g) exposées pendant 20 j à diverses concentrations de cyanure à 13°C dans l'essai 1.

Jour d'échantillonage	Conc. de HCN ($mg \cdot L^{-1}$)	Échantillon (N)	Moyenne de glycogène $mg \cdot g^{-1}$ de foie frais (E.T.)		Limites de confiance à 95 %		Données normalisées par rapport aux témoins
0	0,00	5	60,49	(19,07)	30,17	90,81	100,0
1	0,00	5	113,32	(12,78)	97,43	129,21	100,0
	0,01	5	115,97	(40,79)	65,27	166,68	102,3
	0,02	5	96,63	(37,53)	49,98	143,29	85,3
	0,03	5	105,38	(28,19)	70,33	140,44	93,0
3	0,00	5	119,76	(13,07)	103,52	136,01	100,0
	0,01	5	138,97	(16,91)	117,94	160,00	115,6
	0,02	5	108,10	(29,14)	71,87	144,33	90,3
	0,03	4	92,01	(50,80)	11,24	172,79	76,8
6	0,00	4	123,68	(7,63)	111,55	135,81	100,0
	0,01	4	65,51	(7,65)	53,34	77,68	53,0
	0,02	4	101,45	(26,38)	59,52	143,39	82,0
	0,03	4	61,13	(12,22)	41,70	80,97	49,4
11	0,00	5	70,45	(68,57)	−14,80	155,70	100,0
	0,01	4	70,66	(41,11)	5,25	135,98	100,3
	0,02	4	26,58	(8,95)	12,35	40,81	37,7
	0,03	5	31,04	(22,23)	3,41	58,68	44,1
20	0,00	5	95,48	(56,79)	24,88	166,09	100,0
	0,01	3	105,18	(24,89)	43,39	166,97	110,2
	0,02	5	130,73	(48,30)	80,06	181,41	136,9
	0,03	5	39,87	(34,24)	−8,82	88,56	41,8

était significativement plus élevé ($P < 0,05$) aux trois concentrations d'exposition de HCN (0,01, 0,02, 0,03 $mg \cdot L^{-1}$). Ce phénomène suggère deux hypothèses intéressantes : soit que la biotransformation de HCN en ^{-}SCN est limitée par la cinétique de l'absorption de HCN ou de la mobilisation du soufre, soit que le seuil au-delà duquel l'excès de ^{-}SCN est excrété ait été atteint. Pour les petites truites, les données insuffisantes ne permettent pas de tirer de conclusion sur la cinétique.

Il est donc maintenant bien établi qu'au cours d'expositions prolongées au cyanure à une concentration minimale de 0,01 $mg \cdot L^{-1}$ de HCN, le poisson est soumis à un deuxième agent chimique, le ^{-}SCN. Les implications physiologiques de cette accumulation seront discutées plus bas.

Cytochrome oxydase

La cytochrome oxydase a été l'objet de nombreuses études sur sa nature biochimique et biophysique ainsi que ses réactions avec le cyanure (Van Buuren *et al.* 1972; Wilson et Erecinska 1979). Bien que l'effet toxique du cyanure soit généralement attribué à l'inhibition de la chaîne respiratoire, aucune mesure d'activité de la cytochrome oxydase chez des poissons exposés *in vivo* au cyanure ne semble encore avoir été publiée. Nous avons démontré que le cyanure provoque une forte inhibition (60−80 %) en moins de 24 h d'exposition à des concentrations sous-létales.

Nos observations montrent que la cytochrome oxydase n'a jamais été complètement inhibée, même à 0,03 $mg \cdot L^{-1}$ de HCN. C'est ainsi que Harry *et al.* (1946) ont réduit, *in vitro*, l'activité de la cytochrome oxydase du cerveau de rats de 55 à 65 % par l'addition de 3×10^{-7} M et 1×10^{-6} M de HCN. D'autres études semblables, *in vitro* et *in vivo*, sur des tissus de mammifères révèlent aussi une activité résiduelle de 30 à 40 % (Ballantyne *et al.* 1972; Nakamura *et al.* 1977). Nos résultats et les autres mentionnés plus haut correspondent aux travaux de Commoner (1940) qui estimait à 90 % la proportion de la respiration susceptible à l'inhibition par le cyanure.

Nous avons donc observé une inhibition de 60 à 80 % chez des truites de 170 g et de 60 g après moins de 24 h d'exposition à chacune des trois concentrations de cyanure. De plus, cette inhibition s'est maintenue pour le reste de la période expérimentale.

Les niveaux identiques d'inhibition observés aux trois concentrations de cyanure dès le début de l'exposition nous incitent à considérer le modèle toxicocinétique et toxicodynamique du cyanure décrit ci-dessous.

Le cyanure dans l'eau [HCN] pénètre les branchies du poisson et, puisqu'il s'agit d'une molécule à diffusion rapide, on peut s'attendre en principe, qu'à l'équilibre, la concentration de cyanure dans le milieu interne, [HCN libre], s'approchera de celle présente dans l'eau. Évidemment, plus la concentration externe est élevée, plus le [HCN libre] le sera.

Le [HCN libre] est toutefois influencé par deux facteurs : la transformation de CN^{-} en ^{-}SCN par la rhodanase et ses réactions avec diverses metalloporphyrines (la plupart réversibles) incluant la cytochrome oxydase. Dépendant de la concentration de [HCN libre] et de la vitesse de réaction, on aura différents niveaux d'inhibition des enzymes. Or il y a une forte possibilité que les niveaux d'inhibition de cytochrome oxydase reflètent le [HCN libre]. Nous avons soumis les truites à une concentration maximale de 0,03 $mg \cdot L^{-1}$ de HCN dans les bacs, soit 1,1 μM. Or on sait que l'oxydase de bœuf a une K_i pour le cyanure d'environ 1 μM (la K_i est la concentration causant 50 % d'inhibition, Van Buuren *et al.* 1972). On présume que la K_i d'autres espèces animales, incluant la truite, est à peu près la même. Dans ce cas, en supposant un équilibre entre le [HCN] externe et le [HCN libre], la concentration maximale interne serait

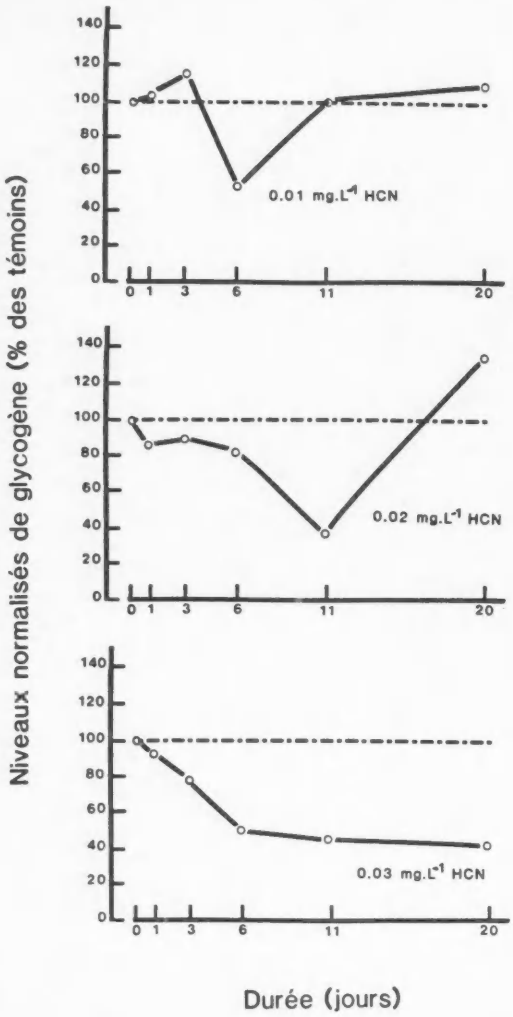
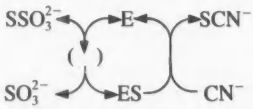


FIG. 3. Niveaux normalisés de glycogène hépatique de truites arc-en-ciel exposées pendant 20 j à différentes concentrations de cyanure, à 13°C. Essai 1.

de 0,5 μM soit à peu près la K_i du cyanure. À cette concentration la cytochrome oxydase n'est jamais complètement inhibée et seule une concentration externe de HCN plus élevée serait létale.

Nous avons observé une inhibition de cytochrome oxydase identique aux trois concentrations de cyanure. Il y a deux explications possibles à ce phénomène. Premièrement, le cyanure, même à 0,01 mg·L^{-1} de HCN, inhiberait complètement la respiration sensible au cyanure, ne laissant que la respiration résistante (±15%) (Commoner 1940). Deuxièmement, la concentration de [HCN libre] devait être la même aux trois concentrations de cyanure, c.-à-d. une concentration minimale effective maintenue par l'action constante de la rhodanase. D'ailleurs nos mesures de $^-$SCN corroborent cette assertion, le $^-$SCN augmentant avec le [HCN] externe. Ceci laisse supposer deux importants phénomènes reliés à l'empoisonnement chronique au cyanure. Premièrement, la transformation du cyanure en $^-$SCN par la rhodanase serait limitée soit par la vitesse de réaction soit par l'apport de soufre sulfane, ou les deux, dans la réaction illustrée ci-dessous (Westley 1981):



Deuxièmement, une concentration constante de [HCN libre] interne indépendante du [HCN] externe serait maintenue aux concentrations sous-létales. Si tel était le cas, toutes les études de toxicité sous-létales dans lesquelles on a observé une relation dose–réponse de croissance, de nage, de reproduction, de respiration, etc. ne refléteraient pas l'effet du cyanure mais plutôt celui du thiocyanate.

Glycogène

Les mesures de glycogène hépatique durant l'intoxication au cyanure suggère une activité de l'effet Pasteur. Cette utilisation de glycogène au cours de l'empoisonnement correspond aux observations de Chaiken *et al.* (1975), de Shaffi et Prasad (1979), et de Kovacs et Leduc (1982). Les poissons utilisent la glycolyse comme source d'ATP de façon continue, du moins à 0,03 mg·L^{-1}. À 0,01 et 0,02 mg·L^{-1} de HCN, le retour des niveaux du glycogène à la normale suggère une reprise de la synthèse d'ATP soit par la chaîne cytochrome ou par une autre route. Ces résultats confirment ceux de Kovacs et Leduc (1982) qui ont aussi observé une baisse, suivie d'un retour à la normale, des taux de glycogène chez des truites arc-en-ciel exposées à 0,015 mg·L^{-1} de HCN, à 12°C. Ces résultats correspondent aux rebonds de croissance observés par Leduc (1966b), Dixon et Leduc (1981), et Kovacs et Leduc (1982), et au rebond de résistance à la toxicité létale du cyanure observé par Dixon et Sprague (1981) après 7 ou 10 j d'exposition au cyanure.

Importance physiologique

Nos résultats indiquent que la truite arc-en-ciel peut détoxifier le cyanure en thiocyanate et l'accumuler dans le plasma à un niveau six fois plus elevé que le font les témoins. Il est aussi apparant que ces poissons peuvent vivre à des concentrations de cyanure de 0,01, 0,02 et 0,03 mg·L^{-1} de HCN avec leur cytochrome oxydase hépatique inhibée entre 60 et 80%. Le glycogène hépatique diminue sous l'influence du cyanure; aux basses concentrations il y a un retour à la normale, mais pas à 0,03 mg·L^{-1} de HCN.

Un poisson exposé au cyanure devient rapidement soumis à un deuxième agent chimique, le thiocyanate, et toute l'interprétation donnée dans le passé sur les effets chroniques du cyanure doit maintenant être revue en fonction du rôle de ce produit de détoxification, particulièrement à cause de ses propriétés de pseudohalogène. En effet, le thiocyanate entre en compétition ionique avec le Cl^- et l'I^- et interfère avec le transport du chlorure au niveau des branchies et avec l'incorporation d'iodure au niveau de la glande thyroïde.

L'absorption active de chlorure en eau douce est inhibée par le thiocyanate chez la truite arc-en-ciel (Kerstetter et Kirschner 1974) et le poisson rouge (*Carrasius auratus*); en eau salée, l'excrétion active de chlorure est également inhibée chez l'anguille (*Anguilla anguilla*) (Epstein *et al.* 1973). Leduc et Chan (1975) ont mis en évidence l'effet nuisible d'une pré-

exposition de truites arc-en-ciel au cyanure sur leur capacité d'osmorégulation et d'ionorégulation en eau douce et salée.

D'autre part le thiocyanate est un agent antithyroïdien en compétition avec le transport actif de l'iodure dans la glande thyroïde des poissons (Singh *et al.* 1977; Eales et Shostak 1983). Chez les mammifères, le cyanure absorbé de façon continue est transformé en $^{-}$SCN et cause une hypothyroïdie. Chez le poisson, cette relation n'a pas encore été établie mais semble très probable malgré l'affirmation de Eales et Shostak (1983): « These data show that KSCN is of no practical value in present population of laboratory trout as a hypothyroid-inducing agent and that considerable care has to be taken in evaluating the effectiveness of thyroidal inhibitors in teleosts ». Ces auteurs n'ont pas mesuré le $^{-}$SCN sérique. Il n'est donc pas possible de comparer les niveaux de $^{-}$SCN à ceux observés au cours de notre étude avec le cyanure.

Il nous semble important d'insister sur le rôle que le thiocyanate provenant du cyanure pourrait avoir sur la fonction thyroïdienne du poisson, et secondairement sur la pituitaire. La glande thyroïde joue un rôle primordial sur la physiologie du poisson. Les altérations de croissance, de respiration, d'osmorégulation et de reproduction causées par les expositions prolongées au cyanure (Leduc 1984) ne sont pas sans suggérer une interférence de $^{-}$SCN au niveau de la glande thyroïde.

L'hypothyroïdisme pourrait aussi avoir des effets néfastes sur la glande pituitaire du poisson à cause d'une surstimulation de sécrétion de TSH. Ceci expliquerait une réduction du potentiel reproducteur de *Jordanella floridae* exposé au cyanure (Cheng et Ruby 1981) ou du poisson chat, *Heteropneustes fossilis*, injecté de KSCN (Singh *et al.* 1977). Par contre, une réduction des fonctions reproductrices pourrait favoriser la croissance somatique (Cheng 1978; Lundqvist 1980).

Cette étude a démontré la nécessité de mesurer *in vivo* l'inhibition enzymatique avant de conclure à l'effet biologique sur la seule base de la nature chimique d'un polluant toxique. Dans le cas présent le thiocyanate doit aussi être considéré. Nous avons montré qu'il n'y a pas de relation dose–réponse cyanure–cytochrome oxydase. De ce fait, il semble bien évident que la relation dose–réponse des effets sous-létaux du cyanure (croissance, reproduction, développement, etc.) (Leduc 1984, p. 215) illustre plutôt la relation dose–réponse du thiocyanate. Toute l'interprétation écotoxicologique du rôle du cyanure devra, en conséquence, être repensée en fonction du thiocyanate qui est un polluant intimement lié à la pollution par les cyanures (Huiatt *et al.* 1983).

Remerciements

Ce projet de recherche a été réalisé grâce à une subvention d'équipe du programme FCAC du ministère de l'Éducation du Québec; nous en sommes reconnaissants. Nous remercions aussi D^{r} Y. Chaubey du Département de mathématiques de l'Université Concordia pour son assistance dans l'analyse statistique.

Références

Ballantyne, B., S. P. Boardman, J. Bright, D. J. Coffee, T. D. Webber et P. Williams. 1972. Tissue cyanide concentrations and cytochrome oxidase activities in experimental cyanide poisoning. Br. J. Pharmacol. 44: 382–383.

Ballantyne, B. 1977. In vitro production of cyanide in normal human blood and the influence of thiocyanate and storage temperature. Clin. Toxicol. 11: 173–193.

Barrett, M. D. P., J. C. Alexander et D. C. Hill. 1978. Effects of dietary cyanide on growth, and thiocyanate levels in blood and urine of rats. Nutr. Rep. Int. 18: 413–420.

Bourdoux, P., F. Delange, F. Gérard, M. Mafuta, A. Hanson et A. M. Ermans. 1978. Evidence that cassava ingestion increases thiocyanate formation: A possible etiologic factor in endemic goiter. J. Clin. Endocrinol. Metab. 46: 613–621.

Chaiken, R., P. Dwight et T. C. Detwiler. 1975. Regulation of platelets phosphorylase. Biochim. Biophys. Acta 403: 315–325.

Chandra, H. B., N. Gupta, S. K. Bhargava, S. H. Clerk et P. N. Mahendra. 1980. Chronic cyanide exposure—a biochemical and industrial hygiene study. J. Anal. Toxicol. 4: 161–165.

Cheng, S. K. 1978. Chronic effects of cyanide on reproduction and development of American flagfish (*Jordanella floridae*). Thèse de maîtrise, Univ. Concordia, Montréal (Québec). 79 p.

Cheng, S. K. et S. M. Ruby. 1981. Effects of pulse exposure to sublethal levels of hydrogen cyanide on reproduction of American flagfish. Arch. Environ. Contam. Toxicol. 10: 105–116.

Commoner, B. 1940. Cyanide inhibition as a means of elucidating the mechanisms of cellular respiration. Biol. Rev. Camb. Philos. Soc. 15: 168–201.

Cooper, T. G. 1977. The tools of Biochemistry. John Wiley and Sons. Toronto. 423 p.

Dixon, D. G. et G. Leduc. 1981. Chronic cyanide poisoning of rainbow trout and its effects on growth, respiration and liver histopathology. Arch. Environ. Contam. Toxicol. 10: 117–131.

Dixon, D. G. et J. B. Sprague. 1981. Acclimation induced changes in toxicity of arsenic and cyanide to rainbow trout. J. Fish Biol. 18: 579–589.

Doudoroff, P. 1976. Toxicity to fish of cyanides and related compounds; a review. Ecol. Res. Ser. EPA 600/3-76-038. Office of Res. and Devel., Environ. Res. Lab., U.S. Environmental Protection Agency, Duluth, MN. 154 p.

Doudoroff, P. 1980. A critical review of recent literature on toxicity of cyanides to fish. American Petroleum Institute, Washington, DC. 71 p.

Eales, J. G. et S. Shostak. 1983. Influence of potassium thiocyanate on thyroid function of rainbow trout, *Salmo gairdneri*. Gen. Comp. Endocrinol. 31: 451–456.

Epstein, H., J. Forrest, A. Epstein et P. Silva. 1973. Thiocyanate inhibition of Cl^{-} transport. Chloride transport and its inhibition in gills of seawater teleosts. Bull. Mt. Desert Isl. Biol. Lab. 13: 32–33.

Good, C. A., H. Kramer et M. Somogyi. 1933. The determination of glycogen. J. Biol. Chem. 100: 485–491.

Harry, G. A., J. Tepperman et O. Bodansky. 1946. A spectrophotometric study of the competition of methemoglobin and cytochrome oxidase for cyanide in vitro. J. Biol. Chem. 163: 641–647.

Huiatt, J. L., J. E. Kerrigan, F. A. Olson et G. L. Potter [éd.]. 1983. Cyanide from mineral processing. Workshop Proceedings, Feb. 2–3, 1982, Salt Lake City. Utah Mining and Mineral Resources Research Institute, College of Mines and Mineral Industries, Salt Lake City, UT. 108 p.

Kerstetter, T. H. et L. B. Kirschner. 1974. HCO_3 dependent ATPase activity in the gills of rainbow trout (*Salmo gairdneri*). Comp. Biochem. Physiol. 48B: 581–589.

Kovacs, T. G. et G. Leduc. 1982. Sublethal toxicity of cyanide to rainbow trout (*Salmo gairnderi*) at different temperatures. Can. J. Fish. Aquat. Sci. 39: 1389–1395.

Lambert, J. L., J. Ramasamy et J. V. Poukstelis. 1975. Stable reagent for colorimetric determination of cyanide by modified Konig reactions. Anal. Chem. 47: 916–918.

Leduc, G. 1966a. Une bouteille à débit constant pour petits volumes de liquides. Nat. Can. (Qué.) 93: 61–64.

Leduc, G. 1966b. Some physiological and biochemical responses of fish to chronic poisoning by cyanide. Thèse doctorale, Oregon State University, Corvallis, OR. 146 p.

Leduc, G. et K. S. Chan. 1975. The effects of chronic cyanide poisoning on the tolerance of rainbow trout to varying salinity, p. 118–125. *Dans* T. C. Hutchison [éd.]. Water Pollution Research in Canada. Inst. Env. Studies, Univ. de Toronto, Toronto (Ont.)

Leduc, G., R. C. Pierce et I. R. McCracken. 1982. The effects of cyanides on aquatic organisms with emphasis upon freshwater fishes. Associate Committee on Scientific Criteria for Environmental Quality. Conseil national de recherches du Canada, Ottawa (Ont.). N° 19246. 139 p.

Leduc, G. 1984. Cyanides in water: Toxicological significance. *Dans* L. A. Weber [éd.]. Aquatic Toxicology. Vol. 2. Raven Press, New York, NY. 230 p.

Leitritz, E. et R. C. Lewis. 1976. Feeding practices in trout and salmon culture: (hatchery method). Dept. of Fish and Game, State of California. 197 p.

Lindman, H. R. 1974. Analysis of variance in complex experimental designs. W. H. Freeman, San Francisco, CA. 352 p.

LUNDQVIST, H. 1980. Influence of photoperiod on growth of Baltic salmon parr (*Salmo salar* L.) with special reference to the effect of precocious sexual maturation. Can. J. Zool. 58: 940–944.

MONTGOMERY, R. 1957. Determination of glycogen. Arch. Biochem. Biophys. 67: 378–386.

NAKAMURA, S., T. SHIN, Y. HIROKATA ET A. SHIGEMATSU. 1977. Inhibition of mitochondrial respiration by sodium nitroprusside and the mechanism of cyanide liberation. J. Anaesth. 49: 1239–1241.

SCHIEVELBEIN, H., R. BAUMEISTERY ET R. VOGEL. 1969. Comparative investigation on the activity of thiosulphate–sulfur transferase. Naturwissenschaften. 56: 416–417.

SHAFFI, S. A. ET D. Y. PRASAD. 1979. Effect of sodium cyanide on tissue energy reserve in a freshwater catfish *Clarias batrachus* (LINN). Curr. Sci. 48: 829–830.

SIDO, B. ET A. KOJ. 1972. Separation of rhodanese and thiosulphate reductase activities in carp liver extracts. Acta Biol. Cracov. Ser. Zool. 15: 97–103.

SINGH, R., R. B. RAIZADA ET T. P. SINGH. 1977. Effects of some antithyroid drugs on the pituitary–thyroid gonad axis in a freshwater catfish *Heteropneustes fossilis* (Bloch). Gen. Comp. Endocrinol. 31: 451–456.

SMITH, L. 1955. Cytochrome a, a_1, a_2, and a_3, p. 732–744. *Dans* J. P. Colowick et N. O. Kaplan [éd.]. Methods in Enzymology. Vol. II. Academic Press, New York, NY.

SOLOMONSON, L. P. 1981. Cyanide as a metabolic inhibitor, p. 29–49. *Dans* B. Vennesland, E. E. Conn, C. J. Knowles, J. Westley et F. Wissing [éd.]. Cyanide in Biology. Academic Press Inc., Londres.

SPRAGUE, J. B. 1973. The ABC's of pollutant bioassay using fish, p. 6–30. *Dans* Biological methods for the assessment of water quality. ASTM STP 528. American Society for Testing and Materials, Philadelphia, PA.

VAN BUUREN, K. J. H., P. NICHOLLS ET B. F. VAN GELDER. 1972. Biochemical and biophysical studies on cytochrome aa_3. VI reaction of cyanide with oxidized and reduced enzyme. Biophysica Acta 256: 258–276.

VENNESLAND, B., E. E. KNOWLES, J. WESTLEY ET F. WISSING [ÉD.]. 1981. Cyanide in Biology. Academic Press Inc., Londres. 548 p.

WAY, J. L. 1981. Pharmacologic aspects of cyanide and its antagonism, p. 29–49. *Dans* B. Vennesland, E. E. Conn, C. J. Knowles, J. Westley et F. Wissing [éd.]. Cyanide in Biology. Academic Press Inc., Londres.

WESTLEY, J. 1981. Cyanide and sulfane sulfur, p. 61–76. *Dans* B. Vennesland, E. E. Conn, C. J. Knowles, J. Westley et F. Wissing [éd.]. Cyanide in Biology. Academic Press Inc., Londres.

WILSON, D. F. ET M. ERECINSKA. 1979. Cytochrome *c* oxidase. The primary regulatory site of mitochondrial oxidative phosphorylation, p. 315–318. *Dans* T. E. King, Y. Orri, B. Chance et K. Okunuki [éd.]. Cytochrome oxidase. Elsevier/North-Holland Biomedical Press, New York, NY.

WOLFF, J. 1964. Transport of iodide and other anions in the thyroid gland. Physiol. Rev. 44: 45–90.

YONETANI, T. 1966. Cytochrome oxidase from beef heart muscle, p. 14–20. *Dans* A. C. M. Maely [éd.]. Biochemical preparation. Vol. II. Wiley, New York, NY.

Estimating the Abundance of Stone-dwelling Organisms: A New Method

Frederick J. Wrona

Aquatic Ecology Group, Division of Ecology, Department of Biology, University of Calgary, Calgary, Alta. T2N 1N4

Peter Calow

Department of Zoology, University of Sheffield, Sheffield, England, G.B. S10 2TN

Ian Ford

Department of Statistics, University of Glasgow, Glasgow, Scotland, G.B. G12 8QQ

Donald J. Baird

Aquatic Ecology Group, Division of Ecology, Department of Biology, University of Calgary, Calgary, Atla. T2N 1N4

and Lorraine Maltby

Department of Zoology, University of Sheffield, Sheffield, England, G.B. S10 2TN

Wrona, F. J., P. Calow, I. Ford, D. J. Baird, and L. Maltby. 1986. Estimating the abundance of stone-dwelling organisms: a new method. Can. J. Fish. Aquat. Sci. 43: 2025–2035.

A system of stratified sampling has been developed which allows the estimation of population densities of organisms which adhere to, or are associated with, stones in both lentic and lotic habitats, using the stones themselves as the primary sampling units. Knowing the stone profile of the habitat (i.e. the mean count, relative frequency, or mean surface area of the stones occurring within a size class), and using the appropriate statistical equations, an estimate of the mean number of individuals occurring within a predefined stone size class can be obtained along with its associated error estimates. Densities of the organisms can be expressed in terms of either a standardized unit area of substratum, average stone size in the habitat, or unit area of stone surface. The statistical theory and computational equations for each sampling method are presented, together with worked examples of the application of the methods in the field. The new sampling protocols are superior to conventional quadrat techniques in population size estimation because (1) the derived density and error estimates account for sources of variation arising from both the heterogeneity of the habitat and the spatial dispersion of the organisms; (2) physical disturbance to the habitat during the sampling program is minimal, and (3) the procedures allow simultaneous quantitative measures of both the density and spatial dispersion of a population.

Nous avons mis au point un système d'échantillonnage stratifié qui permet d'estimer la densité des populations d'organismes qui adhèrent ou sont associées aux roches dans des habitats tant lentiques que lotiques, en utilisant les roches elles-mêmes comme unité primaire d'échantillonnage. Connaissant le profil de l'habitat (cest-à-dire le nombre moyen de roches, leur fréquence relative ou la superficie moyenne des roches se trouvant dans une classe de tailles), et en utilisant les équations statistiques appropriées, on peut obtenir une estimation du nombre moyen d'individus se trouvant dans une classe de tailles de roches définie à l'avance ainsi que les estimations d'erreur connexes. On peut exprimer la densité des organismes soit en fonction d'une unité normalisée de superficie du substrat, soit en fonction de la taille moyenne des roches dans l'habitat, soit par unité de superficie de la surface des roches. Nous présentons la théorie statistique et les équations ayant servi pour chaque méthode, ainsi que des exemples sur l'application de ces méthodes sur le terrain. Les nouveaux protocoles d'échantillonnage sont supérieurs aux méthodes traditionnelles faisant appel aux quadrats pour l'estimation de détail des populations, pour les raisons suivantes : 1) les estimations de la densité et de l'erreur tiennent compte de sources de variations suscitées à la fois par l'hétérogénéité de l'habitat et par la dispersion spatiale des organismes; 2) la perturbation physique de l'habitat pendant l'échantillonnage est minimale; 3) ces méthodes permettent de mesurer simultanément la densité et la dispersion d'une population.

Received January 29, 1986
Accepted June 26, 1986
(J8665)

Reçu le 29 janvier 1986
Accepté le 26 juin 1986

One of the most difficult ecological parameters to measure is the size or density of natural populations, yet such information is crucial for an understanding of population dispersion, dynamics, and demography. This problem is particularly acute in aquatic habitats where the populations can occur on substrates of various shapes and sizes. Existing techniques (time-limited, quadrat, artificial substrate, individual stone sampling) with their associated difficulties have been reviewed in detail (Macan 1958; Calow 1972; Resh 1979; Downing 1984; Peckarsky 1984) and here are only described briefly.

Time-limited sampling suffers heavily from bias related not only to the experience and effort of the investigator, but also to the conditions (e.g. weather) at the time of sampling, and can usually only give crude estimates of densities of populations relative to each other (relative abundance). Artificial substrates do not provide the heterogeneity of natural microhabitats which many organisms experience. Heterogeneity of natural substrates also complicates the interpretation of fixed-area (quadrat) sampling procedures, since one unit of area in one part of the habitat may not contain the same quantity and type of habitat as another part. Thus, the most promising sampling unit is the individual stone, since it is a discrete natural unit to sample (Calow 1972) and many macroinvertebrate species are physically associated with the surfaces of stones. However, since natural stones differ in size and shape and hence in their effective habitat, some allowance must be made for this in correcting counts of individuals per stone to a standard unit of measure.

Our new method of sampling stone-dwelling organisms uses individual stones as the sampling units but, unlike previous stratified sampling techniques, allows for the calculation of variance and standard error estimates that take into account the sources of variation arising from both the physical heterogeneity of the habitat and the spatial dispersion of the organisms among stone size classes.

The technique will first be described, and then the appropriate statistical procedures will be presented, followed by worked examples of case studies and an evaluation of the effects of various parameters (i.e. stone sample size, dispersion pattern of the animals among stone size classes, constant versus proportional sampling protocol with respect to the stone profile) on the derived statistics.

General Description of Technique

Estimates of the population sizes of stone-dwelling organisms are made by using the stones themselves as the sampling units. A predetermined number of stones is chosen within a defined size class and the numbers of organisms found per stone recorded. To translate counts per stone to a measure of density applicable to a particular site, information must be obtained on the patterns of abundance of the stones occurring within the habitat. This requires counting and categorizing a random sample of stones into size classes based on easily measured parameters such as length, breadth, or plan area. Once stone size classes have been defined, size class specific weighting factors can be derived and used to calculate organism population density and error terms. Weighting factors are expressed as either mean counts, relative frequencies, or mean surface areas of the stones occurring within a defined size class as described below. It is important to note that the density units and error estimates obtained in this way depend upon the choice of weighting factor and this, in turn, depends on the ecological question being asked.

The overall population density of a stone-dwelling organism can be estimated using the general relationship

$$(1) \quad d = \sum_{i=1}^{k} y_i \bar{x}_i$$

where d = mean density of organisms (mean number per unit habitat), y_i = stone weighting factor of the ith stone size class (mean count, relative abundance, or surface area of stones), $\bar{x}_i$ = mean number of organisms per stone in the ith stone class, and k = total number of stone size classes. Population density is calculated by weighting the mean organism counts obtained for each stone size class with the appropriate weighting factor and summing across all stone size categories.

The calculation of the density estimates using Equation 1 is straightforward. However, each density estimate for a given organism has two sources of error, one from estimating the stone weighting factor and the other from estimating the mean counts of organisms per size class of stone. These error terms must be combined to calculate the total variance of the final weighted mean density estimate.

Assuming that x_i and y_i are random variables, elementary probability theory yields (after Meyer 1970)

$$(2) \quad V(d) = \sum_{i=1}^{k} V(y_i\bar{x}_i) + \sum_{i=1}^{k-1} \sum_{j=i+1}^{k} 2\,\mathrm{COV}(y_i\bar{x}_i, y_j\bar{x}_j)$$

where $V(d)$ = total variance of the weighted mean density estimate, $V(y_i\bar{x}_i)$ = joint variance of stone and mean organism estimates of the ith stone size class, and 2 COV $(y_i\bar{x}_i, y_j\bar{x}_j)$ = covariance correction between the ith and jth stone size classes.

We emphasize that since $V(d)$ represents the average variance of estimates and not observations, the standard error of the estimated mean density equals the square root of $V(d)$. Therefore, approximate 95% confidence intervals are calculated by multiplying $\sqrt{V(d)}$ with the appropriate normal distribution (z) value.

To calculate the variance of the estimated mean density ($V(d)$), variability of the specific weighting factor for each stone size class must be estimated (see Statistics section).

Choice of Weighting Factor

Three different methods are available for deriving the stone weighting factors (y_i in Equations 1 and 2) from the habitat. These methods determine the units of measure of the final mean density and associated error estimates.

Mean Count

The weighting factor is based on a stone profile of the habitat expressed as the mean count of each stone size class per unit area of substrate. It is estimated by taking replicate, random quadrats of known size and categorizing into definable size classes using suitable measures and counting the stones found in each size class. The mean numbers and variance of the stones within a predefined size class are required for subsequent calculations. For example, if $\bar{n}_s$, $\bar{n}_m$, and $\bar{n}_L$, respectively, denote the mean number of stones per small, medium, and large stone size categories, substituting into Equation 1 yields

$$(3) \quad d = \bar{n}_s\bar{x}_s + \bar{n}_m\bar{x}_m + \bar{n}_L\bar{x}_L$$

where d (the final derived density) is expressed as the mean numbers of organisms occurring per unit area of bottom.

A major criticism of conventional area-limited sampling (e.g. Surber or cylinder samplers) is that errors are confounded owing to habitat heterogeneity and organism dispersion (Calow 1972; Resh 1979). The variance estimate given by Equation 2 allows these two sources of variability to be separated.

Relative Frequency

An expedient way to estimate the stone profile of a habitat would be to census individual stones (e.g. by taking systematic samples along a line transect) to obtain the relative frequencies (i.e. proportions) of stones within each stone size class. An alternative method of calculating the relative frequency of each stone size class would be to use counts from an area-limited sample (above). In both situations the investigator should ensure that any heterogeneity within the area being sampled (e.g. occurrence of patches of similar sized stones due to sorting) is accounted for. Given that f_s, f_m, and f_L are the relative frequencies of the various stone size categories in the habitat, the weighted density estimate becomes

$$(4) \quad d = f_s\bar{x}_s + f_m\bar{x}_m + f_L\bar{x}_L$$

where d is expressed in terms of mean numbers of individuals per average stone occurring in the habitat.

In practice, relative frequencies would normally be estimated. However, it is possible to enumerate the entire population of stones occurring within certain study areas (e.g. a section of a small narrow stream). In this case the stone weighting factors equal the absolute frequencies of each stone size class and the associated error is zero. Thus, the error estimate of the mean density becomes only a function of the dispersion of the organisms within and between stone size categories.

The simplicity of this approach makes it especially useful in preliminary sampling programs comparing the relative abundances of species populations within and between habitats. Additionally, this method could be used as part of other sampling procedures, such as those involved in quantifying the hydrological characteristics of a watershed (Newbury 1984), providing information on both the biotic and abiotic components of the habitat.

Surface Area

The weighting factor is defined as the mean surface area of the stones occurring within each stone size class. The mean surface area ($\bar{a}_i$) of the stones within the ith stone size class (e.g. $\bar{a}_s$, $\bar{a}_m$, and $\bar{a}_L$ representing mean areas of stones in the small, medium, and large stone size classes, respectively) is obtained by one of the many techniques outlined in Calow (1972). Using the reciprocal of the mean surface area as the weighting factor results in the final density estimate expressed as the mean count per unit stone surface area (e.g. numbers per square centimetre of stone surface). Equation 1 becomes

$$(5) \quad d = (1/\bar{a}_s)\bar{x}_s + (1/\bar{a}_m)\bar{x}_m + (1/\bar{a}_L)\bar{x}_L.$$

This density estimate would be useful in studies on the effective habitat utilization of stone-dwelling organisms.

Statistics

In each of the three sampling methods, $E(x)$ and $V(x)$ denote the population mean and variance, respectively, of variable x, while $\mathrm{COV}(x, y)$ is the covariance between variables x and y (the weighting factor).

Equation 1 gives the general form of the population density estimate for the three sampling methods. To assess the accuracy of any point estimate, it is necessary to calculate the variance of that estimate, with the standard error being the square root of the variance. Using small (s), medium (m), and large (L) stone size classes as examples, substitution into Equation 2 yields

$$(6) \quad V(d) = V(y_s\bar{x}_s) + V(y_m\bar{x}_m) + V(y_L\bar{x}_L) + 2\,\mathrm{COV}(y_s\bar{x}_s, y_m\bar{x}_m) + 2\,\mathrm{COV}(y_s\bar{x}_s, y_L\bar{x}_L) + 2\,\mathrm{COV}(y_m\bar{x}_m, y_L\bar{x}_L).$$

Equation 6 is valid in general, but can be simplified by assuming variables x and y to be statistically independent. This requires the weighting factors and the mean densities of the organisms per stone class to be estimated separately. It also assumes values of x to be mutually independent and that a predetermined number of stones was selected from each size class in their calculation. The predetermined total number of stones can be taken in either a constant or proportional manner with respect to the stone profile. The effects of the choice of sampling protocol on the final density and variance estimates were examined using computer simulation.

With these simplifying assumptions, and using the relationship between small and medium stones as an illustration, the joint variance and covariance terms of Equation 6 is

$$(7) \quad V(y_s\bar{x}_s) = (V(\bar{x}_s) + [E(\bar{x}_s)]^2)\,(V(y_s) + [E(y_s)]^2) - [E(\bar{x}_s)E(y_s)]^2$$

$$(8) \quad \mathrm{COV}(y_s\bar{x}_s, y_m\bar{x}_m) = E(\bar{x}_s)\,E(\bar{x}_m)\,\mathrm{COV}(y_s, y_m).$$

If it can be assumed that the covariance terms among the stone sizes classes are negligible, then these can be ignored in Equation 6, and thus provide a simpler, but less accurate method for the estimation of $V(d)$.

Each stone sampling method requires the calculation of $E(\bar{x}_i)$ and $V(\bar{x}_i)$. These terms refer to the mean numbers and associated variances of the organisms occurring per ith stone size class and are estimated by $\bar{x}_i$ and $s^2(x_i)/N_i$, where $\bar{x}_i$ and $s^2(x_i)$ are the sample mean and variance, respectively, over the N_i replicate counts. Therefore:

$$(9) \quad \bar{x}_i = \sum_{r=1}^{N_i} x_{ir}/N_i$$

$$(10) \quad s^2(x_i) = \sum_{r=1}^{N_i} (x_{ir} - \bar{x}_i)^2/(N_i - 1)$$

$$(11) \quad s^2(x_i) = \frac{\sum_{r=1}^{N_i} x_{ir}^2 - \left(\sum_{r=1}^{N_i} x_{ir}\right)^2 \Big/ N_i}{N_i - 1}$$

where x_{ir} = the organism count for the rth replicate stone taken from the ith stone size class and N_i = total predetermined number of randomly selected stones of size class i.

The remainder of this section presents the notation and necessary stone profile statistical estimates. In all cases a circumflex is used to indicate an estimate. A more thorough theoretical treatment can be found in introductory texts on probability theory (e.g. Meyer 1970).

TABLE 1. Computational formulae of stone profile terms used in Equations 6, 7, and 8 for the mean count method. See text for complete explanation of terminology.

Term	Estimate	Formula
$\hat{E}(y_i)$	$\bar{n}_i$	$\bar{n}_i = \sum_{k=1}^{N_q} n_{ik}/N_q$
$\hat{V}(y_i)$	$s^2(n_i)/N_q$	$s^2(n_i) = \left[\sum_{k=1}^{N_q} n_i^2 - \left(\sum_{k=1}^{N_q} n_i\right)^2 \Big/ N_q\right] / (N_q - 1)$
$\hat{\mathrm{COV}}(y_i, y_j)$	$s^2(n_i, n_j)/N_q$	See below

Covariance computational formulae

$$\hat{\mathrm{COV}}(y_i, y_j) = \mathrm{COV}(\bar{n}_i, \bar{n}_j) = s^2(n_i, n_j)/N_q$$

where

$$s^2(n_i, n_j) = \sum_{k=1}^{N_q} (n_{ik} - \bar{n}_i)(n_{jk} - \bar{n}_j)/(N_q - 1)$$

$$= \frac{\sum_{k=1}^{N_q} n_{ik} n_{jk} - \left[\left(\sum_{k=1}^{N_q} n_{ik}\right)\left(\sum_{k=1}^{N_q} n_{jk}\right)\right] \Big/ N_q}{N_q - 1}$$

where
n_{ik} = number of stones of size class i occurring in quadrat k
n_{jk} = number of stones of size class j occurring in quadrat k
$\bar{n}_i$ = mean number of stones of size class i occurring per unit area
N_q = total number of quadrats taken

TABLE 2. Computational formulae of stone profile terms required in Equations 6, 7, and 8 for the relative frequency method. See text for complete explanation of terminology.

Term	Estimate/formula
$\hat{E}(y_i)$	n_i/N_f
$\hat{V}(y_i)$	$(1/N_f)(n_i/N_f)(1 - (n_i/N_f))$
$\hat{\mathrm{COV}}(y_i, y_j)$	$-(1/N_f)(n_i/N_f)(n_j/N_f)$

where
n_i = frequency of stones sampled of size class i
n_j = frequency of stones sampled of size class j
N_f = total number of stones sampled among all size classes

Mean Count

Assume that the data used to estimate the weighting factors (n_s, n_m, and n_L) are counts of small, medium, and large stones observed in N_q random quadrats. These counts are denoted by (n_{si}, n_{mi}, n_{Li}), where $i = 1,2,\ldots, N_q$. Thus, the weighting factors of Equation 1 become $y_s = \bar{n}_s$, $y_m = \bar{n}_m$, and $y_L = \bar{n}_L$.

Estimates of the means and variances within a stone size class and covariances between the pairs of stone size classes are obtained using the formulae in Table 1. These estimates are used in Equations 6 , 7, and 8, with Equation 6 giving the final variance estimate.

The final density estimate must be presented in terms of the area of the quadrat used for determining the stone weighting factors. Scaling the estimate up or down requires more detailed information on the relationships among quadrat size, the number of quadrats taken, and the associated estimated means and variances of the stones occurring within each size class.

Relative Frequency

Assume that a random sample of N_f stones has been obtained from the stone population and that n_s, n_m, and n_L ($n_s + n_m + n_L = N_f$) are the frequencies observed in the three size categories. The weighting factors of Equation 1 becomes $y_s = n_s/N_f$, $y_m = n_m/N_f$, and $y_L = n_L/N_f$.

Hence, the frequencies n_s, n_m, and n_L have a trinomial distribution and the means, variances, and covariances are calculated using the formulae in Table 2.

If the absolute frequency (f_i) of the stones occurring within the ith stone size class is known (e.g. in a situation where all the stones could be counted), using the relative frequency of each stone class as an estimate is unnecessary, and the general formula will still be appropriate. In this case, f_s, f_m, and f_L will replace $E(y_s)$, $E(y_m)$, and $E(y_L)$ in Equations 6, 7, and 8 and the variance and covariance terms involved with the y_i values become zero. The weighting factors used in the density estimate (Equation 1) become $y_s = f_s$, $y_m = f_m$, and $y_L = f_L$.

TABLE 3. Computational formulae of stone profile terms required in Equations 6, 7, and 8 for the surface area method. See text for complete explanation of terminology.

Term	Estimate	Formula
$\hat{E}(y_i)$	$1/\bar{a}_i + (V(\bar{a}_i)/\bar{a}_i^3)$	$V(\bar{a}_i) = s^2(a_i)/R_i$
		$s^2(a_i) = \dfrac{\sum_{r=1}^{R_i} a_{ir}^2 - \left(\sum_{r=1}^{R_i} a_{ir}\right)^2 \Big/ R_i}{R_i - 1}$
$\hat{V}(y_i)$	$(1/\bar{a}_i^4)V(\bar{a}_i)$	
$\hat{\mathrm{COV}}(y_i, y_j)$	0	Covariance equals 0, since all y's are assumed to be independent

where
a_{ir} = area of rth replicate stone selected from size class i
$\bar{a}_i$ = mean area of stones belonging to size class i
R_i = total number of stones belonging to size class i measured for surface area

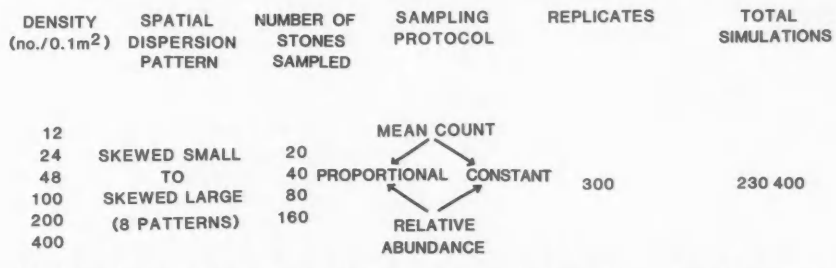


FIG. 1. Combinations and values of variables used in the computer simulations examining the accuracy and precision of the stone sampling system under different sampling protocols.

Surface Area

Assume that random samples of sizes R_s, R_m, and R_L are obtained from three size classes of stones to estimate the average areas $\bar{a}_s$, $\bar{a}_m$, and $\bar{a}_L$. The terms $\bar{a}_s$, $\bar{a}_m$, and $\bar{a}_L$ are estimated using the general formula

$$(12) \quad \bar{a}_i = \sum_{r=1}^{R_i} a_{ir}/R_i$$

where a_{ir} = surface area of the rth replicate stone from size class i and R_i = total number of stones of size class i measured for surface area. Using Equation 1, it is necessary to estimate y_s, y_m, and y_L for $1/\bar{a}_s$, $1/\bar{a}_m$, and $1/\bar{a}_L$, which requires the means and variances for the terms $E(y_i)$ and $V(y_i)$. These estimates, which are used in Equation 6, are obtained using the formulae in Table 3.

When using this method, the covariances will all be zero, since the values of a_i are assumed to be obtained independently. Thus, only approximate means and variances for terms of the form $1/\bar{a}_i$ can be calculated. Since these approximations are based on a Taylor expansion series, they will be valid as long as $\bar{a}_i$ is not too small relative to $V(\bar{a}_i)$. For a derivation of these approximation formulae, see Meyer (1970, p. 139).

Simulation Study

The accuracy and precision of the stone sampling system under different sampling protocols were investigated using Monte Carlo computer simulation. A series of simulations was performed investigating how four independent variables, (1) density of the organisms, (2) spatial dispersion of organisms among the stone size classes, (3) total sample size of stones taken, and (4) sampling protocol (i.e. whether mean count or relative frequency was used as the weighting factor and whether the number of stones sampled per stone size class was either constant or proportional to the relative abundance of the stones within the class), jointly influenced the estimated mean density of the organisms and associated error terms. Figure 1 summarizes the range of values used for each variable, and details of the simulation program are given in the Appendix. The program can simulate any predefined substratum profile, and in this case was based on data collected from Big Hill Spring, Alberta. Therefore, in all calculations the values for the stone weighting factors and associated variance terms for each stone size class were kept constant (Table 4).

For each of the 300 simulations per variable combination, the estimated mean density (d, Equation 1), variance of the mean density ($V(d)$, Equation 2), standard error of the estimated mean ($\sqrt{V(d)}$), and relative error (RE) of the estimated mean were calculated, with

$$(13) \quad \mathrm{RE} = \frac{|\text{estimated density} - \text{actual density}|}{\text{actual density}}$$

where estimated density $= d + \sqrt{V(d)}$. An increase in the magnitude of the relative error value indicates a decrease in the accuracy of the true population density estimate. Frequency distributions of relative error for each combination of variables were generated and used to evaluate the various stone sampling methods.

TABLE 4. Stone profile of Big Hill Spring, Alberta, summarized for each sampling method in terms of total number of stones (n_i), mean counts per 0.1 m^2 of bottom ($\bar{n}_i$), relative frequency (proportion) of total profile (n_i/N_f), mean stone surface area ($\bar{a}_i$), and associated variance ($s^2(y_i)$) terms for each ith stone size class. Stone size classes: VS = very small (1−4 cm^2), S = small (4−16 cm^2), M = medium (16−64 cm^2), L = large (64−256 cm^2); N_q = number of 0.1 m^2 quadrats taken, N_f = total frequency of stones, R_i = total number of stones of size class i for which surface areas were calculated. See text for definition of terms and computational equations.

Size class (i)	n_i	Mean count		Relative frequency				Surface area	
		$\bar{n}_i$	$s^2(n_i)$	n_i/N_f		$s^2(n_i/N_f)$		$\bar{a}_i$	$s^2(a_i)$
VS	1364	68.20	956.1684	0.6321	—	0.2325	—	—	—
S	645	32.25	223.7763	0.2989	0.8123[a]	0.4915	0.1524[a]	11.751	23.464
M	134	6.70	14.7474	0.0621	0.1688[a]	0.0582	0.1403[a]	43.887	138.997
L	15	0.75	3.1447	0.0070	0.0189[a]	0.0070	0.0185[a]	133.019	1321.732
		N_q = 20		N_f = 2158, 794[a]				R_i = 25	

[a]Values recalculated omitting VS stone size class.

Although the simulations examined only the mean count and relative frequency methods, the trends obtained in these simulations are generally applicable to the surface area method, since the simulations primarily test how changes in sampling effort and protocol affect the estimate of $\bar{x}_i$ in Equations 1 and 2 under different population dispersion patterns. The errors associated with the surface area method will be dependent upon the choice of technique used to quantify stone surface area (see Calow 1972).

Simulation Results

Similar trends in relative error were found when either mean count or relative frequency was used as the stone weighting factor for all combinations of simulation variables. Hence, only the results using mean count as the weighting factor are presented. Table 5 gives the maximum relative error (mean RE ± two standard deviations) and associated predicted densities for the lower and upper limit of the densities examined (low: 12 individuals/0.1 m^2; high: 400 individuals/0.1 m^2).

Whilst recognizing that the simulations represent particular situations (i.e. Big Hill Spring stone profile divided into four strata), the two major conclusions described have general applicability. Firstly, as expected, there tended to be a net decrease in relative error with increasing stone sample size. However, the magnitude of decrease was related to both the densities of the organisms and their dispersion patterns among the stone size classes. This was true for both the constant and proportional sampling protocols.

At low densities, when the dispersion patterns of the organisms among stone size classes were relatively evenly distributed or skewed towards the more abundant small stones (patterns I−V, Fig. 2), on average a 23% net decrease in relative error occurred with every doubling in the total number of stones sampled. In contrast, when the organisms were skewed in their distribution to the less abundant larger stones (patterns VI−VIII, Fig. 2), the net decrease in relative error averaged only 6%. At high densities, irrespective of the dispersion pattern of the organisms, it was found that usually less than a 5% decrease in relative error occurred with each doubling in the total numbers of stones sampled. Therefore, at high densities, increased sampling effort (i.e. increasing the number of stones sampled) resulted in only a slight decrease in relative error whereas at low densities this resulted in a substantial decrease in relative error.

Secondly, irrespective of choice of weighting factor (mean count or relative frequency), proportional allocation of the sampling effort among stone sizes generally did not substantially decrease the relative error of the density estimates. A decrease of approximately 15% in relative error with proportional sampling occurred only in low-density situations where the animals were aggregated on small stone size categories (patterns I−II). In all other instances, proportional sampling produced similar or less accurate estimates than the constant sampling protocol. Hence, due to its simplicity, the constant sampling protocol is generally recommended over the proportional sampling protocol.

Case Studies

This section gives worked examples of each sampling method using data collected from Big Hill Spring. The stone profile was determined by categorizing and counting the stones within twenty 0.1-m^2 randomly placed quadrats. Stones were divided into size classes on the basis of plan area. Four stone size classes, 1−4, 4−16, 16−64, and 64−256 cm^2, were used. The mean counts, relative frequencies, and variance terms of the stones occurring within each size class were determined independently (Table 4). Although four stone size categories were identified, the taxa used in the worked examples only were associated with stones in the 4−256 cm^2 range and thus only the small (S), medium (M), and large (L) (Table 4) stone size categories were used in calculations.

Mean Count

This method was used in a study of the population dynamics of the caddisfly *Rhyacophila vao* Milne (Trichoptera: Rhyacophilidae). Final larval instars cling to stones allowing stone sampling without loss of individuals. Monthly density estimates were obtained by randomly selecting a constant number of stones within the small, medium, and large stone size classes (N_i = 20). In the example provided, the estimated density of final instar *R. vao* was 6.18 ± 2.83 larvae/0.1 m^2 of stream bottom (Table 6).

Relative Frequency

Kogotus nonus Needham and Claassen (Plecoptera: Perlodidae) is an active predator that is primarily associated with the undersurface of stones and does not necessarily strongly cling to them (Walde 1985). It was thus necessary to use a

TABLE 5. Maximum percent relative error (RE) of the estimated mean organism density with the associated lower (L) and upper (U) limits of the middle 95% range of predicted densities for a given combination of: dispersion pattern (Patterns I–VIII, Figure 2); organism density (Low = 12 individuals/0.1 m^2: High = 400 individuals/0.1 m^2); stone sample size (n); and sampling protocol (constant versus proportional sampling) when mean count is used as the stone weighting factor.

Dispersion pattern	Density	n	Constant			Proportional		
			RE (%)	L	U	RE (%)	L	U
I	Low	20	142	0	29	121	0	27
		40	110	0	25	81	2	22
		80	73	3	21	47	6	18
		160	45	7	17	30	8	16
	High	20	27	292	508	18	328	472
		40	18	328	472	14	344	456
		80	13	348	452	11	356	444
		160	11	356	444	10	360	440
II	Low	20	105	0	25	111	0	25
		40	89	1	23	86	2	22
		80	63	4	20	51	6	18
		160	43	7	17	36	8	16
	High	20	29	284	516	18	328	472
		40	20	320	480	13	348	452
		80	15	340	460	11	356	444
		160	12	352	448	10	360	440
III	Low	20	101	0	24	111	0	25
		40	79	3	21	80	2	22
		80	55	5	19	54	6	18
		160	37	8	16	34	8	16
	High	20	24	304	496	17	332	468
		40	17	332	468	14	344	456
		80	13	348	452	11	356	444
		160	11	356	444	10	360	440
IV	Low	20	76	3	21	83	2	22
		40	58	5	19	68	4	20
		80	44	7	17	47	6	18
		160	33	8	16	38	7	17
	High	20	25	300	500	21	316	484
		40	21	316	484	21	316	484
		80	19	324	476	19	324	476
		160	18	328	472	18	328	472
V	Low	20	81	2	22	99	0	24
		40	58	5	19	92	1	23
		80	39	7	17	56	5	19
		160	27	9	15	36	8	16
	High	20	18	328	472	15	340	460
		40	15	340	460	17	332	468
		80	13	348	452	13	348	452
		160	12	352	448	12	352	448
VI	Low	20	61	5	19	71	3	21
		40	49	6	18	71	3	21
		80	40	7	17	50	6	18
		160	34	8	16	42	7	17
	High	20	30	280	520	30	280	520
		40	29	284	516	30	280	520
		80	27	292	508	29	284	516
		160	27	292	508	28	288	512
VII	Low	20	62	5	19	63	4	20

TABLE 5. (*Concluded*)

Dispersion pattern	Density	n	Constant RE (%)	Constant L	Constant U	Proportional RE (%)	Proportional L	Proportional U
		40	56	5	19	57	5	19
		80	53	6	18	52	6	18
		160	49	6	18	56	5	19
	High	20	47	212	588	46	216	584
		40	45	220	580	45	220	580
		80	44	224	576	44	224	576
		160	43	228	572	44	224	576
VIII	Low	20	58	5	19	66	4	20
		40	53	6	18	61	5	19
		80	51	6	18	54	6	18
		160	48	6	18	58	5	19
	High	20	45	220	580	47	212	588
		40	43	228	572	45	220	580
		80	43	228	572	44	224	576
		160	42	232	568	44	224	576

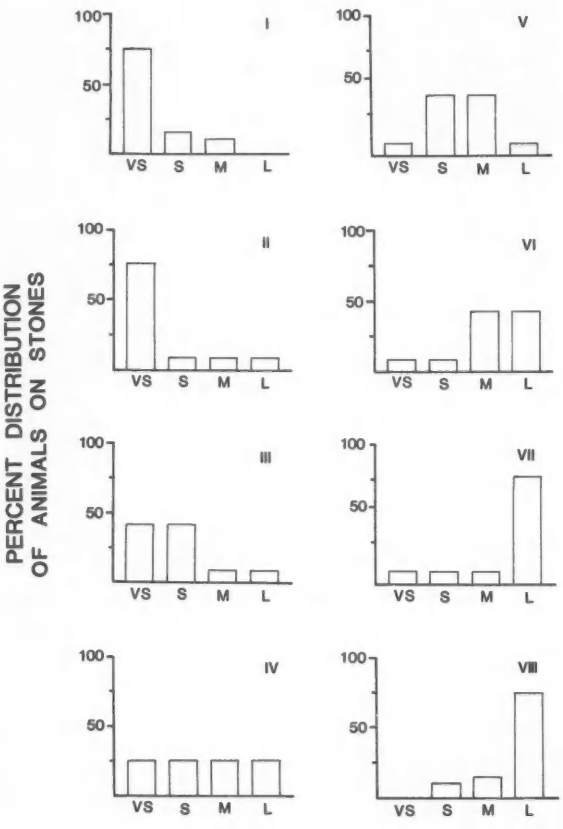


FIG. 2. Dispersion patterns of hypothetical stone-dwelling organisms among the stone size classes as examined in the computer simulations (VS = very small (1−4 cm^2), S = small (4−16 cm^2), M = medium (16−24 cm^2), L = large (64−256 cm^2)).

250-μm catch-net behind each stone to capture any individuals displaced from the stone or the area directly beneath it. (This modification extends the scope of the system to include more mobile macroincvertebrate taxa.) *Kogotus nonus* had an estimated density of 0.29 ± 0.08 individuals/average stone in the habitat (Table 7).

Surface Area

In a study of the microdistribution of the triclad *Polycelis coronata* (Girard) (Turbellaria: Tricladida), an estimate of the density per unit area of the undersurface of stones was required. *Polycelis coronata*, like other triclads, is photonegative and tends to occur on the undersurface of stones during daylight hours.

The average undersurface areas of the stones occurring within the small, medium, and large stone size classes were obtained by randomly selecting 25 stones from each size class and tracing their outline on paper. The areas of these traces were then measured in the laboratory using an area-digitizing system. The mean undersurface areas and variances of the stones occurring within each size class were calculated (Table 4). In this example, *P. coronata* had an estimated density of 0.07 ± 0.02 individuals/cm^2 of undersurface area of stones occurring in the 4−256 cm^2 size range and was markedly aggregated on large stones (64−256 cm^2) (Table 8).

Discussion

Aquatic ecologists have generally selected either area-limited or time-limited sampling methods for estimating the density of natural populations. Although the shortcomings of these approaches have been recognized (Calow 1972; Resh 1979), no alternative sampling procedures which attempt to deal with these shortcomings have been described.

All of the stratified sampling protocols presented in this paper are quantitative and may be applied to a variety of field situations, ranging from simple preliminary/survey sampling programs of species populations (relative frequency method) to those programs requiring more detailed and accurate popu-

TABLE 6. Density calculations of *Rhyacophila vao* in terms of numbers per unit area of stream bottom using the mean count method. Stone size classes: S = small (4−16 cm^2), M = medium (16−64 cm^2), L = large (64−256 cm^2). Stone profile data are from Table 4 and computational formulae can be found in Table 1. See text for definitions of terms.

Term	Estimate	S	M	L
$E(\bar{x}_i)$	$\bar{x}_i$ (Equation 9)	0.1500	0.1500	0.4500
$V(\bar{x}_i)$	$s^2(x_i)/N_i$	0.0067	0.0120	0.0130
N_i		20	20	20
y_i, $E(y_i)$	$\bar{n}_i$	32.25	6.70	0.75
$V(y_i)$	$s^2(n_i)/N_q$	11.1888	0.7374	0.1572
$V(y_i\bar{x}_i)$	Equation 7	7.2951	0.5641	0.0412
		S−M	S−L	M−L
$COV(y_i, y_j)$	Table 1	1.0487	0.1559	0.1645
$COV(y_i\bar{x}_i, y_j\bar{x}_j)$	Equation 8	0.0236	0.0105	0.0111

Density estimate (using Equation 1):

$$d = 32.25(0.1500) + 6.70(0.1500) + 0.75(0.45)$$
$$= 6.18 \text{ individuals}/0.1 \text{ m}^2$$

Variance estimate (using Equation2):

$$V(d) = 7.2951 + 0.5641 + 0.0412 + 2(0.0236) + 2(0.0105) + 2(0.0111)$$
$$= 7.9908$$
$$\text{SE} = \sqrt{7.9908} = 2.83 \text{ individuals}/0.1 \text{ m}^2$$

TABLE 7. Density calculations of *Kogotus nonus* in terms of numbers per average stone using the relative frequency method. Stone size classes: S = small (4−16 cm^2), M = medium (16−64 cm^2), L = large (64−256 cm^2). Stone profile data are from Table 4 and computational formulae can be found in Table 2. See text for definitions of terms.

Term	Estimate	S	M	L
$E(\bar{x}_i)$	$\bar{x}_i$ (Equation 9)	0.2500	0.3500	1.6500
$V(\bar{x}_i)$	$s^2(x_i)/N_i$	0.0099	0.0225	0.2488
N_i		20	20	20
y_i, $E(y_i)$	n_i/N_f	0.8123	0.1688	0.0189
$V(y_i)$	$(1/N_f)(n_i/N_f)(1 - (n_i/N_f))$	0.00019	0.00018	0.00002
$V(y_i\bar{x}_i)$	Equation 7	0.00655	0.00067	0.00015
		S−M	S−L	M−L
$COV(y_i, y_j)$	$-(1/N_f)(n_i/N_f)(n_j/N_f)$	−0.000173	−0.000004	−0.000019
$COV(y_i\bar{x}_i, y_j\bar{x}_j)$	Equation 8	−0.000015	−0.000008	−0.000002

Density estimate (using Equation 1):

$$d = 0.8123(0.25) + 0.1688(0.35) + 0.0189(1.65)$$
$$= 0.29 \text{ individuals/average stone}$$

Variance estimate (using Equation2):

$$V(d) = 0.00655 + 0.00067 + 0.00015 + 2(-0.000015) + 2(-0.000008) + 2(-0.000002)$$
$$= 0.007$$
$$\text{SE} = \sqrt{0.007} = 0.08 \text{ individuals/average stone}$$

lation information (mean count and surface area methods). The methods presented here are superior to more conventional, area-limited population sampling techniques in that they allow the sources of variation due to habitat heterogeneity and organism dispersion to be jointly accounted for in the final density and associated error estimates. By the inherent nature of the proposed sampling systems (i.e. the use of naturally delineated sampling units), it is also possible to obtain simultaneous measures of population density and spatial dispersion. Furthermore, the systems are relatively noninvasive as compared with more conventional sampling techniques (e.g. Surber or cylinder samplers), thereby minimizing physical disturbance to the study area.

Application of the sampling methods proposed requires the

TABLE 8. Density calculations of *Polycelis coronata* in terms of numbers per unit undersurface area of stone using the surface area method. Stone size classes: S = small (4−16 cm^2), M = medium (16−64 cm^2), L = large (64−256 cm^2). Stone profile data are from Table 4 and computational formulae can be found in Table 3. See text for definitions of terms.

Term	Estimate	S	M	L
$E(\bar{x}_i)$	$\bar{x}_i$ (Equation 9)	0.3500	0.4000	3.8000
$V(\bar{x}_i)$	$s^2(x_i)/N_i$	0.0593	0.0284	1.0926
N_i		20	20	20
y_i	$1/\bar{a}_i$ (Table 4)	0.0851	0.0228	0.0075
$E(y_i)$	$1/\bar{a}_i + (V(\bar{a}_i)/\bar{a}_i^3)$	0.0857	0.0229	0.0075
$V(y_i)$	$(1/\bar{a}_i^4)\, V(\bar{a}_i)$	0.000049	0.000001	0.0000002
$V(y_i\bar{x}_i)$	Equation 7	0.00044	0.00002	0.00006
R_i		25	25	25

Density estimate (using Equation 1):

$$d = 0.0851(0.350) + 0.0228(0.400) + 0.0075(3.800)$$
$$= 0.07 \text{ individuals/cm}^2$$

Variance estimate (using Equation 2):

$$V(d) = 0.00044 + 0.00002 + 0.00006 = 0.00052$$
$$\text{SE} = \sqrt{0.00052} = 0.02 \text{ individuals/cm}^2$$

NOTE: Since it is assumed that the weighting factors (y_i's) are independent, there are no covariance terms to calculate.

researcher to have species-specific knowledge of the nature of the relationship between the organism and stone substrate. Obviously, the technique is most easily applied to organisms which are not easily dislodged from stones upon sampling (e.g. triclads, gastropods, leeches, simulids, tube-dwelling chironomids). Extending the use of this technique to include more loosely attached, motile species (e.g. mayflies and stoneflies), although requiring more care in sampling, is possible (see relative frequency case study). There are a wide range of other organisms for which this tecnique would be unsuitable (e.g. benthic zooplankton, oligochaetes, ostracods, tipulids, etc.). It is evident that the proposed sampling methods are not intended for community sampling, where more conventional area-limited techniques would be more suitable.

Although the sampling system was designed and tested for use in aquatic habitats, it is equally applicable to any organism which occurs in discrete habitat islands that can be categorized and enumerated (e.g. aphids on leaves, birds in woodlands). Thus, it is hoped that the sampling methods and statistics described in this paper will offer ecologists practical, alternative approaches for sampling many types of field populations.

Acknowledgments

We thank R. W. Davies, L. R. Linton, and F. E. R. McCauley and three anonymous reviewers for critically reviewing earlier versions of this manuscript. The authors express a special thanks to R. W. J. Dixon for his technical assistance and involvement in writing the simulation programs. This research was supported by a NSERC operating grant to F.J.W. and NERC grants to PC.

References

CALOW, P. 1972. A method for determining the surface areas of stones to enable quantitative estimates of littoral stonedwelling organisms to be made. Hydrobiologia 40: 37−50.

DOWNING, J. A. 1984. Sampling the benthos of standing waters, p. 87−130. *In* J. A. Downing and F. H. Rigler [ed.] A manual on methods for the assessment of secondary productivity in fresh waters. 2nd ed. IBP Handbook 17, Blackwell Scientific Publications, Oxford, UK.

MACAN, T. T. 1958. Methods of sampling the bottom fauna in stony streams. Mitt. Int. Ver. Limnol. 8: 1−21.

MEYER, P. L. 1970. Introductory probability and statistical applications. 2nd ed. Addison-Wesley Publ. Co., London, UK.

NEWBURY, R. W. 1984. Hydrologic determinants of aquatic insect habitats, p. 323−357. *In* V. H. Resh and D. M. Rosenberg [ed.] The ecology of aquatic insects. Praeger Publ., New York, NY.

PECKARSKY, B. 1984. Sampling the stream benthos, p. 131−160. *In* J. A. Downing and F. H. Rigler [ed.] A manual on methods for the assessment of secondary productivity in fresh waters. 2nd ed. IBP Handbook 17, Blackwell Scientific Publications, Oxford, UK.

RESH, V. 1979. Sampling variability and life history features: basic considerations in the design of aquatic insect studies. J. Fish. Res. Board Can. 36: 290−311.

WALDE, S. J. 1985. Invertebrate predator−prey interactions and stream community structure. Ph.D. thesis, University of Calgary, Calgary, Atla.

Appendix: Description of the Simulation Program

The simulations were performed using a Fortran (ansii 77) program which was developed on a Honeywell multics series 68 dps2 computer (University of Calgary). The program simulated a stone substrate having any predefined profile. However, in all the simulations the actual values used as the stone weighting factors and associated variances were based on the Big Hill

TABLE A.1. Average simulated mean ($E(\bar{x})$) and associated variance ($s^2(x)$) of the numbers of animals occurring on a stone for each combination of proportional stone size class representation and organism density used in the computer simulations. Note that the variance is approximately equal to the mean, confirming a random distribution of individuals among the stones within a size class. Stone size classes: VS = 1−4 cm², S = 4−16 cm², M = 16−64 cm², L = 64−256 cm².

Stone size class	Proportional representation (%)	Theoretical density (number/0.1 m²)			
		12		400	
		$E(\bar{x})$	$s^2(x)$	$E(\bar{x})$	$s^2(x)$
VS	76	0.143	0.144	4.513	4.167
	75	0.144	0.150	4.464	4.304
	42	0.081	0.083	2.480	2.264
	25	0.041	0.042	1.478	1.407
	8	0.020	0.020	0.467	0.471
	0	—	—	—	—
S	42	0.164	0.162	5.306	5.440
	25	0.102	0.105	3.167	3.240
	15	0.062	0.062	1.898	1.809
	10	0.041	0.041	1.265	1.246
	8	0.041	0.040	1.025	1.040
M	42	0.760	0.759	25.599	26.172
	25	0.447	0.425	15.214	15.317
	15	0.306	0.319	9.129	8.936
	10	0.140	0.144	6.094	5.915
	8	0.145	0.149	4.870	4.423
L	76	12.246	12.023	413.590	434.860
	75	12.240	11.809	408.200	385.540
	42	6.802	7.020	228.610	231.530
	25	4.075	3.972	133.080	138.430
	8	1.367	1.392	43.527	42.921
	0	—	—	—	—

Spring data (Table 4). Figure 1 summarizes the combinations of sampling methods and variables tested.

A vector consisting of 200 elements represented a substratum having four size classes (50 elements each), with each element of the vector acting as a single stone. A function, which generated uniformly distributed random values in the range 1−200, loaded hypothetical organisms into the vector elements according to the spatial dispersion pattern and density being examined. Six organism density levels, ranging from 12 to 400 individuals/0.1 m², were examined. Within a sampling run, a given organism density was distributed among the four stone size classes (i.e. representing 1−4, 4−16, 16−64, and 64−256 cm² intervals) according to one of eight possible spatial dispersion patterns (Fig. 2). Table A.1 summarizes the average density and associated variance of the numbers of organisms occurring on a stone for each of the different possible proportional representations of individuals among the four stone size classes for the lowest (12 individuals/0.1 m²) and highest (400 individuals/0.1 m²) simulated densities.

Within a single sampling run, a random number function selected a predetermined total number of stones (n = 20, 40, 80, or 160) according to the sampling protocol being investigated (i.e. a constant number of stones being taken per stone size class versus a number that was proportional to the relative abundance of the size class in the habitat). For example, if the total sample size was to be 80 stones and the sampling procedure a constant size relative to the stone profile, 20 stones (vector elements) from each of the four stone size categories were randomly selected. In contrast, when examining the influence of proportional sampling, 63.20% of the 80 stones (i.e. 50 stones) were selected from the 1−4 cm² stone size class, 24 stones (29.89%) from the 4−16 cm² class, 5 stones (6.21%) from the 16−64 cm² class, and 1 stone (0.7%) from the 64−256 cm² class (Note: a minimum of 1 stone was always selected per size class when performing proportional sampling, since the technique requires all strata to be always sampled).

Three hundred replicate sampling runs were conducted for each combination of variables being examined (Fig. 1), with the organism density estimate (d, Equation 1), variance of the estimate ($V(d)$, Equation 2), and relative error the estimate (RE, Equation 13) being calculated in each.

NOTES

Comparison of Continuous and Episodic Exposure to Acidic, Aluminum-Contaminated Waters of Brook Trout (*Salvelinus fontinalis*)

Lisbeth K. Siddens, Wayne K. Seim, and Lawrence R. Curtis

Department of Fisheries and Wildlife, Oak Creek Laboratory of Biology, Oregon State University, Corvallis, OR 97331, USA

and Gary A. Chapman

Western Fish Toxicology Station, United States Environmental Protection Agency, Corvallis, OR 97330, USA

Siddens, L. K., W. K. Seim, L. R. Curtis, and G. A. Chapman. 1986. Comparison of continuous and episodic exposure to acidic, aluminum-contaminated waters of brook trout (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. 43: 2036–2040.

This study demonstrated that repetitive, intermittent exposures to aluminum (Al) under acidic conditions produced greater cumulative mortality and lower growth rates of brook trout (*Salvelinus fontinalis*) than would be extrapolated from continuous exposure experiments. During a 24-d study, exposures were at pH 4.4 or 4.9 with 0.2–1.2 mg Al·L^{-1}. Data were analyzed based on peak and 24-d mean Al concentrations of continuous and intermittent (2-d toxicant, 4-d ambient condition cycle) exposures. Survival was lower in groups exposed to at pH 4.9 than those at 4.4 when intermittently exposed but not when continuously exposed. Growth rates were less for fish continuously exposed at pH 4.4 compared with those at pH 4.9, while intermittent exposures to Al acidity alone did not affect growth. Fish intermittently exposed to Al at pH 4.4 grew faster than those at pH 4.9, indicating greater Al toxicity at the higher pH.

La présente étude démontre que des expositions répétées et discontinues à l'aluminium (Al) en milieu acide se traduisent, chez l'omble de fontaine (*Salvelinus fontinalis*), par une mortalité cumulative plus importante et des taux de croissance inférieurs aux valeurs que l'on obtient par extrapolation des essais d'exposition en continu. Au cours d'une étude de 24 j, des poissons ont été exposés à des teneurs d'Al variant de 0,2 à 1,2 mg·L^{-1}, à des pH de 4,4 ou de 4,9. Les données ont été analysées en fonction d'expositions à des concentrations d'Al maximales de pointes ou moyennes de 24 j en régime continu ou discontinu (cycle de 2 j d'exposition au toxique et de 4 j aux conditions ambiantes). Le taux de survie était inférieur chez les groupes exposés à l'Al au pH 4,9, comparativement au pH 4,4, en régime discontinu mais non en régime continu. Les taux de croissance étaient inférieurs chez les poissons ayant subi une exposition continue à pH 4,4 plutôt qu'au pH 4,9; une exposition discontinue au seul milieu acide n'avait pas d'effet sur la croissance. Les poissons exposés à l'Al en régime discontinu présentaient un taux de croissance plus rapide à pH 4,4 qu'à pH 4,9, ce qui indique une toxicité plus grande de l'Al au pH plus élevé.

Received July 15, 1985
Accepted June 2, 1986
(J8325)

Reçu le 15 juillet 1985
Accepté le 2 juin 1986

Losses of fish and other organisms from natural waters have been correlated with the presence of aluminum (Al) mobilized from soils by acidic storm or snowmelt events (Driscoll et al. 1980; Harriman and Morrison 1982; Gee and Wade 1984; Sharpe et al. 1984). For example, peak total Al concentrations in a southwestern Pennsylvania watershed during rain and snowmelt events were 0.85 mg·L^{-1} in organic soil leachate and 3.59 mg·L^{-1} in mineral soil leachate, while surface flow samples contained only about 0.25 mg·L^{-1} (Sharpe et al. 1984). Low-alkalinity streams in this area were subject to pH lows of 4.4–4.5, peak Al concentration of 1.5 mg·L^{-1}, and contained few or no fish.

The transient nature of acid-related changes in water quality is established for both streams and lakes (Gunn and Keller 1984; Sharpe et al. 1984) and within interstitial water of artificial redds (Lacroix 1985). Mortality of *Salvelinus namaycush* sac fry in test chambers set above the rubble bed of an Ontario Lake was attributed to the longest (5 d) of four snowmelt episodes (Gunn and Keller 1984). Mortality of 18% was primarily associated with maximum surface runoff, peaks of inorganic Al (0.04–0.05 mg·L^{-1}), and low pH (4.5–5.0). Rubble interstitial waters contained more inorganic Al (0.08 mg·L^{-1}) than ambient waters.

Aluminum toxicity was inversely related to calcium ion con-

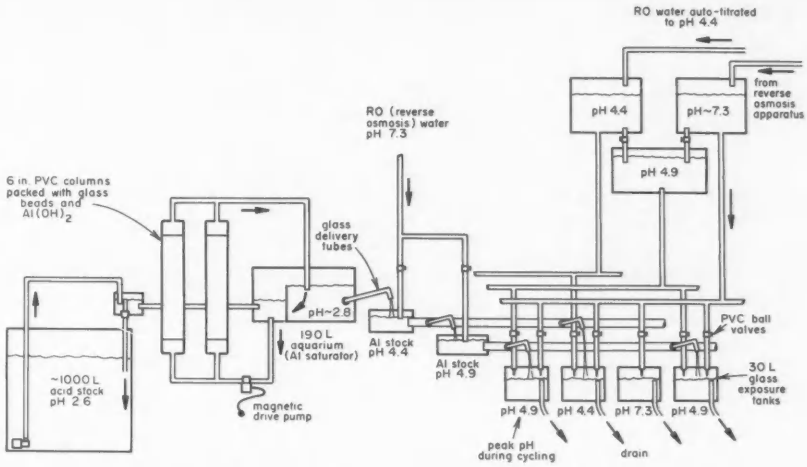


FIG. 1. Simplified drawing of the Al stock solution generator, the distribution system for Al, acid, and dilution waters, and the exposure tanks.

centrations between 0.25 and 2.0 mg·L^{-1} (Brown 1983) and depended on pH, being most toxic at about pH 5.0–5.5 (Baker and Schofield 1982). Life cycle stage also influences response to Al exposure. Tolerance to Al decreased with age from eggs to postlarval brook trout (*Salvelinus fontinalis*) (Baker and Schofield 1982) and continued to decrease over the first year of life for brown trout (*Salmo trutta*) (Brown 1983).

While Al is a major determinant of response by aquatic organisms in acidic environments, the utility of continuous exposure experiments in understanding episodic exposures has received scant investigation. When comparisons were based on mean toxicant concentrations, intermittent were often more toxic than continuous exposures (Brown et al. 1969; Coffin et al. 1977; Ingersoll and Winner 1982; Seim et al. 1984; Curtis et al. 1985). We therefore compared toxicity of continuous and cyclic exposures to acidified, Al-contaminated solutions in laboratory studies of juvenile brook trout. Growth and survival were chosen as meaningful, sensitive toxicity end points.

Methods

Juvenile brook trout were hatched, spawned, and raised at the Western Fish Toxicology Laboratory, USEPA, Corvallis, OR, where experiments were conducted. Because environmental acidification was reported to be of primary concern in soft, low-alkalinity water (Haines 1981), raw water from the Willamette River was treated by filtration and reverse osmosis, which reduced measured materials in the water by about 60%. Raw Willamette River water exhibits mean alkalinity and hardness of about 23 and 22 mg·L^{-1}, respectively. Additional characteristics of Willamette River water reported by Chapman (1978) indicated relatively low levels of complex-forming substances such as organic carbon (3.4 mg·L^{-1}) and sulfate (6.6 mg·L^{-1}).

Treated water was softened to maintain hardness and alkalinity at 8–10 mg·L^{-1} and calcium at about 2 mg·L^{-1}. Temperature was kept at 17.0°C (SEM = 0.18, N = 14). Softened water was acidified to pH 4.40 with H_2SO_4 using a Beckman automatic titrator and diluted with softened control water (pH 7.3) to obtain pH 4.90 (Fig. 1). Aluminum was added to the system by flowing acidified water (pH 2.60, calibrated manually) through two 183 × 15 cm PVC cylinders. Each column was packed with 1.5-cm glass beads mixed with $Al(OH)_3 \cdot nH_2O$ gel (J. T. Baker purified grade). The columns were attached to a recycling saturator (Gingerich et al. 1979) maintaining a mean Al concentration of 9.81 mg·L^{-1} (SEM = 0.43, N = 24) and pH at 2.80. The Al-saturated water was diluted with control water to obtain an Al stock of pH 4.40, part of which was again diluted with control water to obtain a pH 4.90 solution. In this way we were able to produce specific Al concentrations without changing the pH from 4.40 or 4.90.

Flow rate into each exposure tank was maintained at 250 mL·min^{-1} (±10%). Nominal Al concentrations for pH 4.4 were 0, 0.4, 0.8, and 1.2 mg·L^{-1} and for pH 4.9 were 0, 0.2, 0.4, and 0.8 mg·L^{-1}. For pH 7.30 (control), no Al was added. Each combination was run both continuously (24 d) and intermittently (2 d of exposure and then 4 d at control) throughout the test, resulting in 17 test environments. Aluminum samples were taken daily in the saturator and constant exposure tanks. The intermittent cycles were measured for both pH and Al at 15- to 30-min intervals from the time exposure flows started or ended to that time where Al concentrations appeared to stabilize and pH reached background level (Fig. 2) and daily thereafter. The time course of Al concentration for intermittent exposure cycles generally followed the form expected from estimates of the time for partial volume replacement based on exchange rate and tank volume (Sprague 1969). The pH time course was altered perhaps by buffering capacity and added $Al(OH)_3$ (Fig. 2). Four such 6-d cycles for each intermittent treatment were completed in the 24-d study.

All Al samples were analyzed by atomic absorption (graphite furnace) spectroscopy and expressed as total Al. To determine dissolved Al in samples, aliquots were either filtered through a 0.45-μm filter or centrifuged at 10 000 rpm for 10 min. No difference was found between these and unfiltered or uncentrifuged samples. Thus, nearly all the Al was in a dissolved form although individual Al species were not determined. Because of low organic carbon concentrations estimated for treated water, much of the Al was probably inorganic complex ions of hydroxide and other ligands as described by Robertson

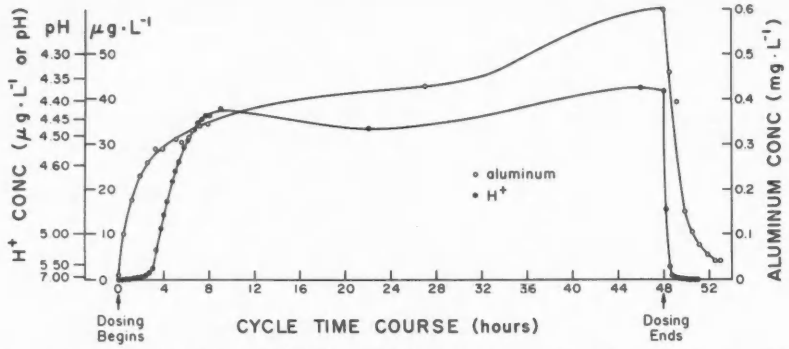


FIG. 2. Concentration of Al and H^+ during the 48-h dosing period of one 6-d cycle. Concentrations returned to control levels for the 4 d following dosing.

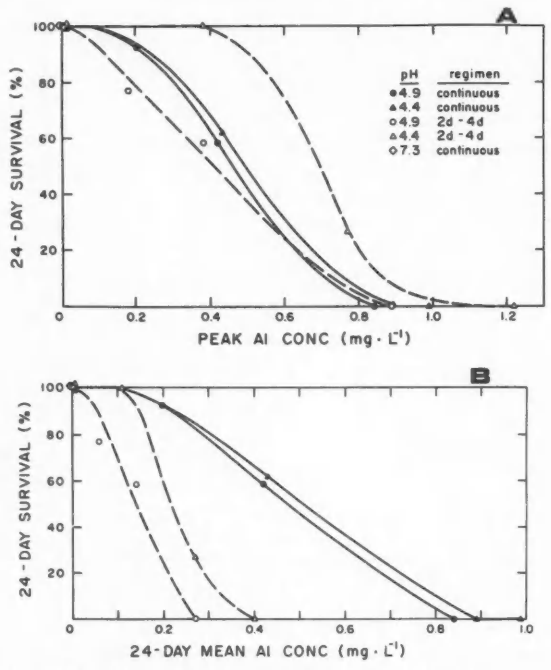


FIG. 3. The 24-d survival of juvenile brook trout at (A) peak exposure concentrations of Al and (B) Al concentration expressed as a 24-d mean measured in the exposure water throughout the experiment. Continuous exposures are shown by solid symbols and solid lines; intermittent exposures (open symbols and broken lines) were four cycles of 2-d exposure to increased Al concentration followed by a 4-d exposure to control (pH 7.3, no Al). Each point represents a sample of 20 fish.

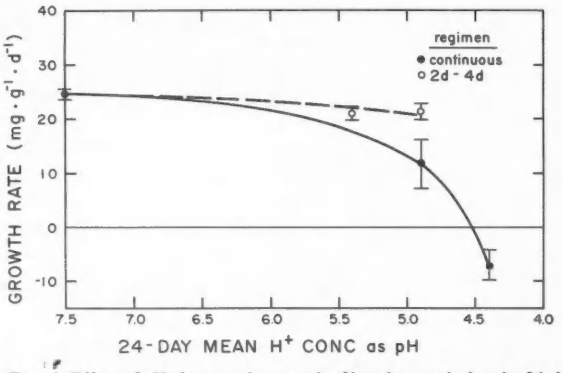


FIG. 4. Effect of pH alone on the growth of brook trout during the 24-d test. Mean H^+ concentration was determined from measurements of the exposure water taken throughout the test then expressed as pH. The solid line shows continuously exposed fish; the broken line shows intermittently exposed fish (2 d of acid followed by 4 d of control water).

and Hem (1969).

Ten days prior to the test, fish were anesthetized with MS-222 and branded with marking tools chilled in an acetone – dry ice mixture using a modification of the technique described by Groves and Novotny (1965). Fish were put into exposure tanks with control water and fed Oregon Moist Pellet ad libitum until 48 h prior to the test at which time food was withheld. Twenty-four hours before the test, fish were again anesthetized with MS-222 to record brand and measure initial wet weight. Mean wet weight was 2.42 g (SEM = 0.20). Initial dry weight was determined from a sample of 10 branded fish not used in the test. These were killed in a solution of MS-222, weighed, dried at 70°C for 96 h, and reweighed to determine initial percent dry weight of test fish. Twenty fish were placed in each exposure tank and fed Oregon Moist Pellet daily at 12%·d^{-1}. Rations were adjusted each day for mortalities, and uneaten food was siphoned daily. At termination of the test, all surviving fish were killed in MS-222, dried at 70° for 96 h, and weighed. Growth was calculated as average relative growth rate over the experimental period based on the change in dry weights (Warren 1971):

$$\text{Growth rate} = \frac{w_2 - w_1}{0.5(w_1 + w_2)(t_2 - t_1)}$$

where w = dry weight and t = time.

Results

The 24-d survival was 100% at pH 4.4 or 4.9 without Al regardless of exposure regimen (Fig. 3). Peak Al of about 0.2 mg·L^{-1} or higher (0.4 or higher for intermittent pH 4.4) significantly reduced survival below control levels (Fig. 3A). For continuous exposures, no fish survived beyond the third

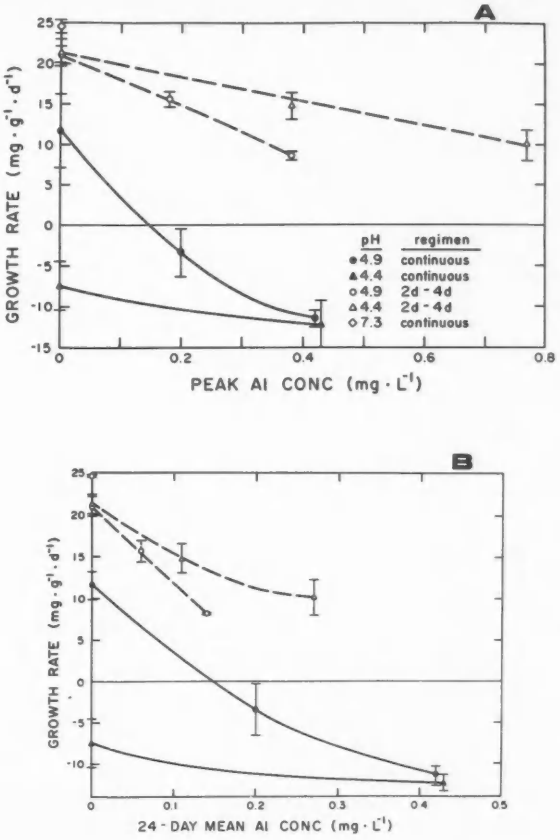


FIG. 5. Growth rate of brook trout exposed to various Al concentrations expressed as (A) peak concentration determined from peaks reached throughout the 24 d and (B) mean concentration determined from measurement of the exposure water throughout the 24-d test. Continuous exposures are shown by solid lines; intermittent exposures (2-d Al exposure, 4-d control) are shown by broken lines. Analysis of variance indicated a significant concentration effect ($p < 0.05$) in Fig. 5A.

test day at or above 0.83 mg $Al \cdot L^{-1}$. There was little difference in survival for continuous exposure at pH 4.4 and 4.9 for any Al concentration. Intermittent exposure, however, did result in greater Al toxicity at pH 4.9 than at 4.4.

When Al concentration was expressed as the 24-d mean, it was shown to be more toxic for the intermittent than for the continuous regimen (Fig. 3B). This trend has also been demonstrated for ammonia and copper (Brown et al. 1969; Seim et al. 1984). When Al concentration was expressed as peak concentration, survival of continuously exposed fish was again greater than for intermittently exposed fish at pH 4.9 but less than the survival for intermittently exposed fish at pH 4.4 (Fig. 3A). Thus, for fish intermittently exposed to Al at pH 4.9, there was no survival advantage derived from the four 4-d recovery periods under control conditions.

Growth was reduced in comparison with controls (pH 7.3) for continuous exposures to pH 4.9 or 4.4 without addition of Al (Fig. 4). Growth rates were negative at continuous pH 4.4 exposure. Lower pH levels (pH 4.0 and 4.2) were lethal in our preliminary tests (data not shown). Growth of juvenile brook trout was not reduced compared with controls by cycling 2-d acid pulses at peak pH levels as low as 4.4 followed by 4 d at ambient pH (24-d mean pH 4.8) (Fig. 4).

The addition of Al to low-pH water further decreased growth rates of brook trout which survived 24 d in all treatments except continuous exposure to pH 4.4 (compare Fig. 4, 5A, 5B at pH 4.4). However, for the intermittent regimen, Al exposure at pH 4.4 reduced growth in a concentration-dependent manner (Fig. 5). For continuous pH 4.4, the growth reduction appeared to be due largely to acid rather than Al (Fig. 4, 5). At pH 4.4, acidity alone caused weight loss. Because this loss presumably approaches some maximum temperature-dependent rate, the addition of Al perhaps could not greatly accelerate the rate of loss over that caused by pH alone. Survival, however, decreased significantly with increased Al at pH 4.4 (Fig. 3). The effect of Al was more severe at pH 4.9 than at 4.4 for both regimens. This was consistent with other research which indicated maximal Al toxicity near pH 5 (Driscoll et al. 1980; Schofield and Trojnar 1980).

Discussion

Results for nonlethal continuous exposure to Al were consistent with those of Baker and Schofield (1982) who found that 0.1 mg $Al \cdot L^{-1}$ at pH 4.9 and 5.5 reduced growth but not survival of brook trout sac fry. Their results were partially explained in terms of synergism of acid and metal toxicities. At pH 4.2, inclusion of up to 0.5 mg $Al \cdot L^{-1}$ produced less mortality of brook trout embryos (but not fry) than acid alone. Muniz and Leivestad (1980) reduced plasma chloride loss in brown trout at pH 4.0 by addition of 0.4 mg $Al \cdot L^{-1}$. These kinds of data indicated not only synergistic toxic actions of acidity and Al but also antagonistic interactions under certain conditions. Generally, Al toxicity might be expected to predominate around pH 5.0 and acid toxicity nearer pH 4.0.

Of particular interest was the similar or greater mortality for fish exposed intermittently to Al at pH 4.9 as compared with continuous exposures. This suggested that episodic Al exposure of brook trout at critical pH levels was more toxic than could be extrapolated from continuous exposure studies. The toxicity of 48-h Al pulses at near maximally synergistic pH was apparently too marked for 4-d periods under control conditions to allow recovery. Earlier research indicated the gill as the principal "target organ" for Al toxicity in fishes (Muniz and Leivestad 1980; Schofield and Trojnar 1980). Immediate toxic events were epithelial necrosis and clogging of the gills with mucus, and osmoregulatory dysfunction and impaired gas exchange were perhaps secondary effects. Inhibition of gill carbonic anhydrase and Na^+/K^+-ATPase activities (Staurnes et al. 1984) probably contributed to physiologic disturbances, while contact toxicity of Al precipitated upon gill surfaces probably played a predominant role in toxicity (Schofield and Trojnar 1980). Under nonlethal conditions, effects on growth were less than expected for intermittently exposed fish (compare Fig. 3B, 5B). We saw intermittently exposed fish consume food between acidic Al pulses, while continuously exposed fish ate infrequently, often mouthing and dropping food pellets. Continuous exposure to depressed pH with or without Al was more detrimental to growth rate under conditions not lethal in our 24-d experiment. Indeed, Rogers (1984) found reduced growth of juvenile brook trout continuously exposed to pH 5.3 for 4 wk.

In other work, repetitive pH depressions (i.e. pH 4.0–4.5) were not toxic to brook and rainbow trout (*Salmo gairdneri*) at durations shorter than 24 h until months of cyclic exposure (Curtis et al., unpubl. data). Considering the magnitude of the environmental acidification problem and the complexities of pH–Al synergism, further investigation of these time–concentration relationships appears to be warranted.

Acknowledgments

This study was supported by USEPA CR-810157-20, National Acid Precipitation Program. Preliminary results of this research were presented at the 1985 Society of Toxicology Meeting (San Diego, CA). This is technical paper No. 7597 of the Oregon State University Agricultural Experiment Station.

References

BAKER, J. P., AND C. L. SCHOFIELD. 1982. Aluminum toxicity to fish in acidic waters. Water Air Soil Pollut. 18: 289–309.

BROWN, D. J. A. 1983. Effects of calcium and aluminum concentrations on the survival of brown trout (*Salmo trutta*) at low pH. Bull. Environ. Contam. Toxicol. 30: 582–587.

BROWN, V. M., D. H. M. JORDAN, AND B. A. TILLER. 1969. The acute toxicity to rainbow trout of fluctuating concentrations and mixtures of ammonia, phenol, and zinc. J. Fish Biol. 1: 1–9.

CHAPMAN, G. A. 1978. Toxicities of cadmium, copper, and zinc to four juvenile stages of chinook salmon and steelhead. Trans. Am. Fish. Soc. 107(6): 841–847.

COFFIN, D. L., D. E. GARDNER, G. I. SIDORENKO, AND M. A. PINIGIN. 1977. Role of time as a factor in the toxicity of chemical compound in intermittent and continuous exposures. Part II. Effects of intermittent exposure. J. Toxicol. Environ. Health 3: 821–828.

CURTIS, L. R., W. K. SEIM, AND G. A. CHAPMAN. 1985. Toxicity of fenvalerate to developing steelhead trout following continuous or intermittent exposure. J. Toxicol. Environ. Health 15: 445–457.

DRISCOLL, C. T. JR., J. P. BAKER, J. J. BISOGNI, AND C. L. SCHOFIELD. 1980. Effect of aluminum speciation on fish in dilute acidified waters. Nature (Lond.) 284: 161–164.

GEE, A. S., AND K. R. WADE. 1984. The effects of acidification on the ecology of streams in the upper Tywi catchment in west Wales. Environ. Pollut. 35: 135–157.

GINGERICH, W. H., W. K. SEIM, AND R. D. SCHONBROD. 1979. An apparatus for the continuous generation of hydrophobic chemicals. Bull. Environ. Contam. Toxicol. 23: 685–689.

GROVES, A. B., AND A. J. NOVOTNY. 1965. A thermal-marking technique for juvenile salmonids. Trans. Am. Fish. Soc. 94: 386–389.

GUNN, J. M., AND W. KELLER. 1984. Spawning site water chemistry and lake trout (*Salvelinus namaycush*) sac fry survival during spring snowmelt. Can. J. Fish. Aquat. Sci. 41: 319–329.

HAINES, T. A. 1981. Acidic precipitation and its consequences for aquatic ecosystems: a review. Trans. Am. Fish. Soc. 110: 669–707.

HARRIMAN, R., AND B. R. S. MORRISON. 1982. Ecology of streams draining forested and non-forested catchments in an area of central Scotland subject to acid precipitation. Hydrobiologia 88: 251–263.

INGERSOLL, C. G., AND B. W. WINNER. 1982. Effect on *Daphnia pulex* (De Greer) of daily pulse exposures to copper or cadmium. Environ. Toxicol. Chem. 1: 321–327.

LACROIX, G. L. 1985. Survival of eggs and alevins of Atlantic salmon (*Salmo salar*) in relation to the chemistry of interstitial water in redds in some acidic streams of Atlantic Canada. Can. J. Fish. Aquat. Sci. 42: 292–299.

MUNIZ, I. P., AND H. LEIVESTAD. 1980. Toxic effects of aluminum on the brown trout (*Salmo trutta*) L., p. 320–321. *In* D. Drablos and A. Tollan [ed.] Proceedings of the International Conference on the Ecological Impact of Acid Precipitation. SNSF Project, Oslo, Norway.

ROBERTSON, C. E., AND J. D. HEM. 1969. Solubility of aluminum in the presence of hydroxide, fluoride, and sulfate. U.S. Geological Survey Water-Supply Paper 1827-C, U.S.G.S.

ROGERS, D. W. 1984. Ambient pH and calcium concentration as modifiers of growth and calcium dynamics of brook trout (*Salvelinus fontinalis*) in acidified waters, p. 341–366. *In* T. Y. Toribara, M. W. Miller, and P. E. Morrow [ed.] Polluted rain. Plenum Press, New York, NY.

SCHOFIELD, C. L., AND J. R. TROJNAR. 1980. Aluminum toxicity to brook trout (*Salvelinus fontinalis*) in acidified waters, p. 341–3562. *In* T. Y. Toribara, M. W. Miller, and P. E. Morrow [ed.] Polluted rain. Plenum Press, New York, NY. 500 p.

SEIM, W. K., L. R. CURTIS, S. W. GLENN, AND G. A. CHAPMAN. 1984. Growth and survival of developing steelhead trout (*Salmo gairdneri*) continuously or intermittently exposed to copper. Can. J. Fish. Aquat. Sci. 41: 433–438.

SHARPE, W. E., D. R. DEWALLE, R. T. LEIBFRIED, R. S. DINICOLA, W. G. KIMMEL, AND L. S. SHERWIN. 1984. Causes of acidification of four streams on Laurel Hill in southwestern Pennsylvania. J. Environ. Qual. 13(4): 619–631.

SPRAGUE, J. B. 1969. Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. Water Res. 3: 793–821.

STAURNES, M., T. SIGHOLT, AND O. B. REITE. 1984. Aluminum toxicity to brook trout (*Salvelinus fontinalis*) in acidified waters, p. 341–3562. *In* T. Y. Toribara, M. W. Miller, and P. E. Morrow [ed.] Polluted rain. Plenum Press, New York, NY. 500 p.

WARREN, C. E. 1971. Biology and water pollution control. W. B. Saunders Co., Philadelphia, PA. 434 p.

Variation in Major Ion Concentration of *Cambarus robustus* and *Orconectes rusticus* Following Exposure to Low pH

Lois Hollett, Michael Berrill, and Locke Rowe

Watershed Ecosystems Program, Trent University, Peterborough, Ont. K9J 7B8

Hollett, L., M. Berrill, and L. Rowe. 1986.Variation in major ion concentration of *Cambarus robustus* and *Orconectes rusticus* following exposure to low pH. Can. J. Fish. Aquat. Sci. 43: 2040–2044.

Cambarus robustus is more tolerant of low environmental pH than *Orconectes rusticus* and this tolerance reflects a difference in ion regulation physiology. Chronic exposure (96 h) of the acid-tolerant *C. robustus* to pH 3.8 soft water did not significantly change haemolymph $[Na^+]$ or $[Ca^{2+}]$ of the adults or total body $[Na^+]$ of the juveniles relative to the control (pH 6.5). In contrast, the intolerant *O. rusticus* showed a significant decrease in $[Na^+]$ and increase in $[Ca^{2+}]$ in adult haemolymph (Wood and Rogano. 1986. Can. J. Fish. Aquat. Sci. 43: 1017–1026)

and an increase in total body [Na^+] of stage III juveniles following acute exposure to pH 3.8 compared with the pH 6.5 control.

Cambarus robustus est une espèce plus tolérante d'un faible pH environnemental que *Orconectes rusticus*, et cette tolérance reflète une différence dans la physiologie de la régulation des ions. Une exposition chronique (96 h) de *C. robustus*, qui tolère un milieu acide, à une eau douce présentant un pH de 3,8 n'a pas modifié de façon notable l'ion [Na^+] ni le [Ca^{+2}] dans l'hémolymphe des adultes ni le [Na^+] de l'ensemble du corps des juvéniles par rapport au groupe témoin (pH 6,5). Par opposition, l'espèce peu tolérante *O. rusticus* a montré une nette baisse de [Na^+] et une augmentation de [Ca^{+2}] dans l'hémolymphe des adultes (Wood et Rogano. 1986. Can. J. Fish. Aquat. Sci. 43: 1017–1026) et une augmentation de [Na^+] de l'ensemble du corps chez les juvéniles de stade III à la suite d'une exposition aiguë à un pH de 3,8 par opposition au groupe témoin soumis à un pH de 6,5.

Received December 12, 1985
Accepted June 25, 1986
(J8612)

Reçu le 12 décembre 1985
Accepté le 25 juin 1986

Crayfish vary in their tolerance of pH stress with respect to species and life history stage. Adult and stage III juvenile *Cambarus robustus* are more tolerant of laboratory and in situ low pH than those of *Oronectes* species, and this variation in tolerance is reflected in species distribution with respect to pH (Berrill et al. 1985). Gradual acidification of Experimental Lakes Area Lake 223 to pH 5.1 resulted in a progressive decrease in population numbers of *Orconectes virilis* due to failed recruitment and possibly the disruption of ion regulation (Schindler et al. 1985). Further experiments indicate that juvenile *O. virilis* (France 1984), *O. rusticus*, and *O. propinquus* (Berrill et al. 1985) are more sensitive to low pH than adults. While the major effect of environmental acidity is severe haemolymph acidosis, an accompanying disruption of ion regulation in adults of several intolerant crayfish species, including the intolerant *O. rusticus*, has been reported (Appelberg 1985; Morgan and McMahon 1982; Wood and Rogano 1986). Typically, [Na^+] and [Cl^-] decreases while [Ca^{2+}] increases in the haemolymph. These results appear to be independent of water hardness in adult crayfish (Morgan and McMahon 1982; Wood and Rogano 1986). Similar physiological studies with tolerant species are required to establish that interspecific differences in pH tolerance observed in the field are correlated with differences in physiology. To date, no physiological studies have been done on the adults or juveniles of acid-tolerant crayfish.

In this study we examine the effect of acid exposure on haemolymph [Na^+] and [Ca^{2+}] in the relatively acid-tolerant *C. robustus* adult and on the total body [Na^+] of stage III juveniles of *O. rusticus* and *C. robustus*. Stage III juveniles were tested in varying [Ca^{2+}] in order to determine the effect of ambient [Ca^{2+}] on exposure to low pH. Results of Wood and Rogano (1986) for adult *O. rusticus* are used for comparative purposes with adult *C. robustus*.

Given the small body size of juvenile crayfish, whole body analysis is an attractive method for measuring ion concentration. Individual variation in carapace and claw development and the localization of Ca^{2+} in these structures meant that changes in [Ca^{2+}] could not be accurately measured in juveniles.

Methods

Experimental animals and water — In our laboratory experiments we used sexually mature, intermoult adults and stage III juveniles. Early stage III juveniles are particularly attractive for laboratory experiments, for they are capable of independent existence prior to their dispersal, they carry yolk reserves for the first 1–2 wk of that stage and therefore do not need feeding, and they can be collected in relatively large numbers from a single female. All adult crayfish were collected approximately 24 h prior to experimentation. *Cambarus robustus* were collected from Gull River (Victoria County) and *O. rusticus* was collected from Thompson's Creek (Peterborough County), both hardwater streams in southern Ontario with similar water chemistry to that of the Otonabee River (Table 1).

Water used for the exposure of adult *C. robustus* was laboratory-constituted soft water made by adding a specified quantity of each element (usually in a compound form) to deionized water (Table 1). In order to establish a possible [Ca^{2+}] mitigating effect, three natural water sources were selected for exposure of stage III juveniles: the hardwater Otonabee River, the relatively soft Kosh Lake water, and the very soft Plastic Lake water (Table 1). Water pH was established and maintained within 0.2 pH unit using 1 *N* H_2SO_4 and 1 *M* KOH. pH 3.8 was selected as the test pH with an intermediate pH of 5.0 for juvenile exposure. Although pH 3.8 is lower than levels found in acid waters, this level allows comparison with other studies (i.e. Wood and Rogano 1986: pH 4.0; Morgan and McMahon 1982: pH 3.8). All water was decarbonated, aerated continuously, and maintained at 15°C.

Adult exposure — Twenty *C. robustus* adults (male and females) were collected in late May and were placed in the test water at a pH of 6.5 for a 7-d acclimation period. Water was renewed on the fourth day. Test chambers were static 10-L aquaria, each holding 10 crayfish. Following acclimation, 10 individuals were exposed to pH 3.8 for 4 d. The remaining 10 crayfish were left at pH 6.5 for the same 4-d period to act as a control group. Chambers were checked twice daily for pH fluctuations or mortality. After the exposure period, a 0.1-mL haemolymph sample was drawn from near the basipodite of a walking leg of each crayfish using a 1-mL syringe. Haemolymph samples were transferred to 10 mL of deionized water and diluted to a detectable level (0.006–0.026 μmol/mL for Na^+ and 0.025–0.100 μmol/mL for Ca^{2+}). In order to suppress sodium and calcium ionization, potassium (0.05 mmol/mL) was added to each sample, and chemical interference in the determination of [Ca^{2+}] was prevented by the addition of strontium chloride (0.06 mmol/mL). Samples were then analysed for [Na^+] and [Ca^{2+}] by atomic emmission and atomic absorption, respectively, on a Varian 375 atomic

TABLE 1. Major ion concentration of the four experimental water sources in mmol/L (mg/L in parentheses).

Water source	Ca^{2+}	Na^{+}	Cl^{-}	K^{+}	Mg^{2+}	SO_4^{-}	Al ($\times 10^{-3}$)
Artificial soft	0.05 (2.35)	0.04 (0.92)	0.18 (6.38)	0.01 (0.39)	0.01 (0.24)	0.04–0.09 (3.83–8.62)	—
Plastic Lake	0.05 (2.35)	0.02 (0.46)	0.01 (0.35)	0.01 (0.39)	0.02 (0.49)	0.07 (6.70)	0.37–3.70 (10–100)
Kosh Lake	0.18 (7.21)	0.10 (2.30)	0.06 (2.13)	0.03 (1.17)	0.04 (0.97)	0.07 (6.70)	—
Otonabee River	0.95 (38.01)	0.13 (2.99)	0.10 (3.55)	0.03 (1.17)	0.03 (0.73)	0.10 (9.60)	—

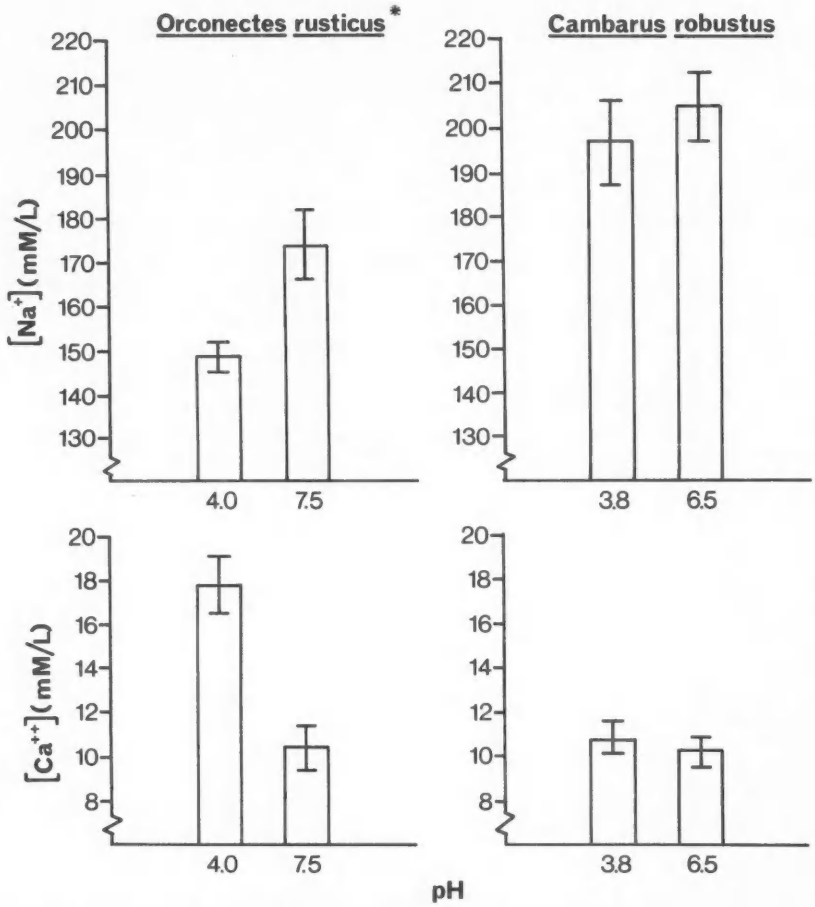


FIG. 1. Haemolymph $[Na^{+}]$ and $[Ca^{2+}]$ in *O. rusticus* and *C. robustus* following 96-h exposure to three pH levels. Bars represent means ± 95% confidence intervals. (*Results for *O. rusticus* are taken with permission from Wood and Rogano 1986)

absorption spectrophotometer. Actual concentrations were determined comparing sample results to a similar range of known N.B.S. standards.

Juvenile exposure — Female *O. rusticus* with eggs were collected in May 1984 and kept in static 10-L aquaria in each of the three water types at pH 6.5 until the young crayfish had moulted to stage III (approximately 2 wk). Water was renewed approximately every 5 d. Female *C. robustus* with stage II young were collected in mid-August 1984 and similarly kept until the juveniles moulted to stage III (approximately 4 d). Stage III juveniles of each species were exposed to three pH

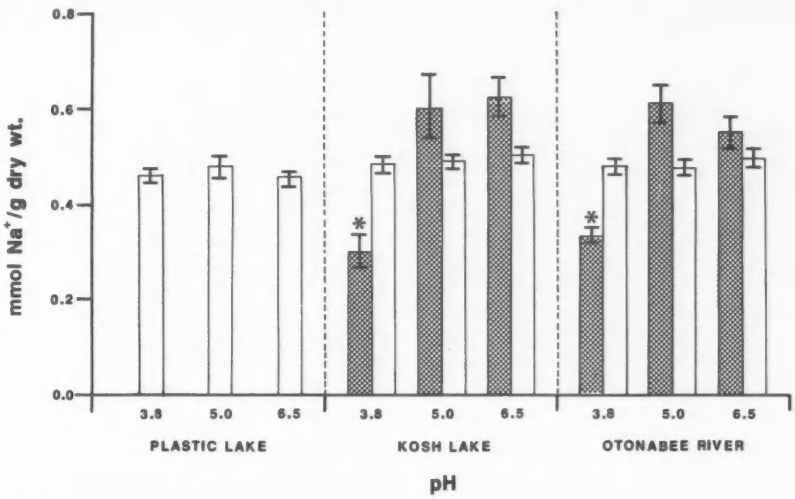


FIG. 2. Concentration of total body [Na^+] in stage III juvenile *O. rusticus* (shaded bars) and *C. robustus* (open bars) following 96-h exposure to three pH levels. Bars represent means ± 95% confidence intervals. *Result for 48 h.

levels (3.8, 5.0, and 6.5) in each water type for 4 d. Enough *O. rusticus* juveniles were obtained to allow us to expose a total of 450 individuals in replicates of 25 per 2-L container of water, at each pH in each water type. The limited number of stage III *C. robustus* which we obtained allowed us to test only 25 in each treatment. pH was checked and maintained daily, and any dead juveniles were removed. After 4 d, surviving juveniles were dried to a constant weight at 60°C. However, in Plastic Lake water, *O. rusticus* mortality was too great to allow the exposure to last for more than 24 h at all pH levels or for more than 48 h in the other two water types at pH 3.8. *Orconectes rusticus* juveniles were digested in groups of three by refluxing with 1 mL of concentrated HNO_3 over a hot plate for 0.5 h (one blank was run with each set of digestions). Each solution was then diluted with 0.1 *N* HCl such that final solutions were within the detectable range (0.006–0.026 μmol/mL). Potassium (0.05 mmol/mL) was again added to each sample, and the emission of each solution was measured for [Na^+] on a Varian 375 atomic absorption spectrophotometer. *Cambarus robustus* were treated in a similar manner except they were digested in pairs because of their larger size.

Statistical analysis — For both experiments, sample means were compared using Student's t-Test. Differences were considered significant at the 95 percent confidence level.

Results and Discussion

Adult *C. robustus* appeared unaffected by exposure to low pH. Four-day exposure of adult *C. robustus* to pH 3.8 soft water [Ca^{2+}] = 0.05 mmol/L) had no significant effect on haemolymph [Na^+] and [Ca^{2+}] relative to control animals (pH 6.5; Fig. 1). In comparison, 4-d exposure of adult *O. rusticus* to pH 4.0 soft water ([Ca^{2+}] = 0.1 mmol/L) resulted in significant loss in haemolymph [Na^+] and significant gain in haemolymph [Ca^{2+}] (Wood and Rogano 1986).

Stage III juvenile *C. robustus* total body [Na^+] remained remarkably consistant in all treatments and no mortality occurred, while juvenile *O. rusticus* suffered significant losses of Na^+ and increased mortality in treatments of low pH and low [Ca^{2+}], independent of low pH (Fig. 2). Juvenile *O. rusticus* did not survive the (96-h) exposure to pH 3.8 in either the hard water of Otonabee River (10% mortality) or the relatively soft water of Kosh Lake (15% mortality). Those exposed to pH 3.8 in both hard and soft water lost approximately 50% of their total body [Na^+] after 48 h compared with the controls (pH 6.5) after 96 h, a highly significant difference ($p < 0.01$, Otonabee water; $p < 0.01$, Kosh Lake water). [Na^+] did not change in juveniles exposed to pH 5.0 relative to those exposed to pH 6.5 in these two water types. There was no significant difference between results for Otonabee River and Kosh Lake waters. Only 5 of the 150 stage III *O. rusticus* exposed to the three pH levels in the very soft Plastic Lake water survived for 24 h (1 at pH 3.8, 3 at pH 5.0, and 1 at pH 6.5). Although too few to consider statistically, all five had lost at least 70% of their total body [Na^+]. Although aluminum concentrations are higher in Plastic Lake, increased aluminum concentration at pH 4.5–5.0 has been shown to have no affect on mortality of *O. rusticus* (Berrill et al. 1985). It appears that low ambient [Ca^{2+}], independent of pH level, is lethal to *O. rusticus*, since mortality was equally high at all pH levels in Plastic Lake.

The results reported here clearly illustrate that interspecific differences in low pH tolerance by crayfish reflect differences in their ion regulation physiology. Net losses of Na^+ are due mainly to an inhibition of Na^+ uptake while efflux rates remain largely unchanged (Shaw 1960; Wood and Rogano 1986). Since *C. robustus* showed no change in blood Na^+ concentration, it may be able to maintain Na^+ influx in low pH water. Elevated haemolymph [Ca^{2+}] following low pH exposure has been reported for the intolerant *O. rusticus*, *O. propinquus* (Wood and Rogano 1986), and *Procambarus clarkii* (Morgan and McMahan 1982) and appears to result from dissolution of $CaCO_3$ from the carapace to act as a buffer against blood acidosis which occurs in acid-stressed crayfish (Wood and Rogano 1986; Morgan and McMahon 1982). Since haemolymph [Ca^{2+}] did not increase in *C. robustus*, this may indicate

that this species is also resistant to blood acidosis.

There is growing evidence indicating that *Cambarus* as a genus is tolerant to low pH, while *Orconectes* is not (Berrill et al. 1985), and the data reported here suggest that the difference is one of ion physiology. Evolutionary events may account for these physiological differences between genera. The genera *Cambarus* and *Orconectes* both belong to the relatively recently evolved Cambaridae family of crayfish, but whereas *Orconectes* apparently originated in the central basin of the three great rivers of central North America (the Mississippi, Ohio, and Missouri), *Cambarus* appears to have originated in the mountainous regions of the Southern Appalachians and Ozarks (Hobbs 1942, 1974). The two genera have therefore evolved under quite different conditions, including those of water chemistry. It is possible that *Cambarus*, evolving under the softer water conditions characteristic of mountain streams, evolved ionoregulation mechanisms that preadapted it to withstanding low pH stress more successfully than *Orconectes*.

Acknowledgements

This research was supported by grants from the Natural Sciences and Engineering Research Council of Canada and the Ontario Ministry of the Environment. We thank Chris Wood for allowing us to present his data and for commenting on an early draft of the manuscript and Bruce LaZerte for advice on water chemistry.

References

APPELBERG, M. 1985. Changes in haemolymph ion concentrations of *Astacus astacus* L. and *Pacifastacus leniusculus* (Dana) after exposure to low pH and aluminum. Hydrobiologia 121: 19–25.

BERRILL, M., L. HOLLETT, A. MARGOSIAN, AND J. HUDSON. 1985. Variation in tolerance to low environmental pH by the crayfish *Orconectes rusticus*, *O. propinquus*, and *Cambarus robustus*. Can. J. Zool. 63: 2586–2589.

FRANCE, R. L. 1984. Comparative tolerance to low pH of three life stages of the crayfish *Orconectes virilis*. Can. J. Zool. 62: 2360–2363.

HOBBS, H. H. JR. 1942. A generic revision of the crayfishes of the subfamily Cambarinae (Decapoda, Astacidae) with the description of a new genus and species. Am. Midl. Nat. 28(2): 334–357.

1974. Synopsis of the families and genera of crayfishes. Smithson. Contrib. Zool. 164: 1–32.

MORGAN, D. O., AND B. R. MCMAHON. 1982. Acid tolerance and effects of sublethal acid exposure on ionoregulation and acid–base status in two crayfish *Procambarus clarki* and *Oronectes rusticus*. J. Exp. Biol. 97: 241–252.

SCHINDLER, D. W., K. H. MILLS, D. F. MALLEY, D. L. FINDLEY, J. A. SHEARER, I. J. DAVIES, M. A. TURNER, G. A. LINSEY, AND D. R. CRUIKSHANK. 1985. Long-term exosystem stress: the effects of years of experimental acidification on a small lake. Science (Wash., DC) 228: 1395–1401.

SHAW, J. 1980. The absorption of sodium ions by the crayfish *Astacus pallipes* Lereboullet. II. The effect of the external anion. J. Exp. Biol. 37: 534–547.

WOOD, C. M., AND M. S. ROGANO. 1986. Physiological responses to acid stress in crayfish (*Orconectes*): haemolymph ions, acid–base status, and exchanges with the environment. Can. J. Fish. Aquat. Sci. 43: 1017–1026.

High Precision Microcomputer Based Measuring System for Ecological Research

John C. Roff and Russell R. Hopcroft

Department of Zoology, University of Guelph, Guelph, Ont. N1G 2W1

Roff, J. C., and R. R. Hopcroft. 1986. High precision microcomputer based measuring system for ecological research. Can. J. Fish. Aquat. Sci. 43: 2044–2048.

A semiautomated measuring system is described which allows precise multiple measurements on organisms of biological interest. It consists of a microscope with drawing tube positioned next to a digitizing tablet which is interfaced to a personal computer; a TV camera and monitor are optional additions. Light from an LED fitted cursor on the digitizing pad is focused through the drawing tube and combined with the microscope image. Measurement signals are sent to the computer when the cursor button is depressed. Data storage, calculation, and display can be performed on-line as data are entered. Maximum precision of repeated measurements is ±0.04%; in routine use an accuracy of <±0.25% is achieved. An example of its precision compared with a conventional eyepiece micrometer is given. The system has been used for measurement of a diversity of aquatic particles including phytoplankton, zooplankton, fecal pellets, and stream benthic invertebrates at magnifications from 10 to 1250 power.

Nous décrivons un système de mesure semi-automatique qui permet d'effectuer des mesures multiples et précises sur des organismes intéressant le biologiste. Il se compose d'un microscope avec tube de tracé localisé près d'un numériseur qui possède une interface avec un ordinateur personnel; une caméra de télévision et un écran peuvent y être ajoutés. La lumière provenant d'un curseur à DEL sur le numériseur est focalisée par le tube de traçé et se combine à l'image du microscope. Les signaux de mesure sont envoyés à l'ordinateur lorsque le bouton du curseur est enfoncé. Il est possible d'effectuer l'archivage des données, le calcul et l'affichage en direct à mesure que les données sont entrées. La précision maximum de mesures répétées est de ±0,04 %; dans l'emploi ordinaire, on obtient une précision de <±0,25 %. Nous donnons un exemple de ce degré de précision comparé

à celui d'un micromètre optique conventionnel. Le système a servi à mesurer la diversité des particules aquatiques, notamment le phytoplancton, le zooplancton, les pelotes fécales et les invertébrés benthiques des cours d'eau avec des grossissements allant de 10 à 1 250 fois.

Received December 5, 1985
Accepted June 27, 1986
(J8600)

Reçu le 5 décembre 1985
Accepté le 27 juin 1986

The measurement of organisms (biological particles) has become an increasingly vital task as ecologists strive for improved quantification. This is especially true in research on aquatic ecosystems which are dominated by microscopic particles. With growing emphasis on relationships between size or weight of organisms and their metabolic or ecological role (e.g. Sheldon et al. 1972; Banse and Mosher 1980; Peters 1984), accurate and precise measurements are at a premium.

It is now commonplace to derive weight estimates of organisms from their linear dimensions by some power function (e.g. McCauley 1984). Because length–weight relationships are power functions, small deviations in length measurements produce considerable changes or variance in the dependant weight estimates. Dimensions of individuals must be measured with the highest possible accuracy and precision, and preferably repeated or several body measurements should be made (Pearre 1980; McCauley 1984).

Because of our requirements for biomass determinations on a variety of planktonic particles and stream invertebrates, we have developed a semiautomated measuring system with versatile data storage, manipulation, calculation, and retrieval capabilities. Measurements and calculations of biomass can be made to a very high degree of precision and accuracy. In this note our system is described, and its advantages are compared with other measuring or image analysis systems.

Description

The measuring system consists of a microscope with drawing tube and optional TV camera and monitor. A digitizing tablet with LED cursor is positioned next to the microscope, beneath the drawing tube which looks down onto it, and is connected to a personal computer.

The apparatus as presently configured (Fig. 1) consists of either a Leitz Labovert inverted microscope or a Wild M8 zoom binocular microscope with drawing tube, a Hi-Pad digitizing tablet interfaced to an IBM-PC computer, a Hitachi high resolution TV camera and 17-in. (43-cm) monitor, and various data calculation and file management programs in BASIC.

Any compound or binocular microscope can be used provided that it can be fitted with a drawing tube. Any digitizing tablet with LED cursor can be used provided that it can be interfaced to a PC (e.g. IBM, Apple, etc.) computer. Any size of particle or image which can be visualized in a light microscope can be measured. We have used our measuring system at magnifications from 10 to 1250 power.

The system operates as follows. The object to be measured is placed under the microscope in the normal fashion. The light from the LED cursor is focused by the drawing tube, so that it provides a fine point of light combined with the microscope image. The combined images are then projected onto a black and white or colour TV monitor, as well as onto the ocular lenses.

By adjusting the magnification factor of the drawing tube (we use a factor of 16 or 25×), the working area of the digitizing pad can be made to correspond to either the entire field of view in the microscope or to the entire TV monitor screen. Thus, maximum resolution and precision of measurement can be achieved. Accuracy can be altered simply by increasing microscope magnification. Measurements can be made either whilst viewing through the microscope or viewing the TV screen.

Measurements are made in any direction by moving the LED cursor across the digitizing pad until the red light coincides with one point of the object to be measured. The LED cursor button is pressed and a coordinate signal is sent to the computer. This operation is repeated at another point on the object, a second set of coordinates is sent to the computer, and a measurement is immediately calculated employing a previously calibrated magnification scale. In addition, curved or flexed specimens are measured by summing short linear measurements traced along it.

After identification to taxon or category by the operator, particle type is entered as a code of one or more letters and/or digits or function keys from the PC keyboard. Alternatively, defined sectors of the Hi-Pad itself can be used for this purpose. Replicate measurements on the same particle can be made as fast as the LED cursor can be repositioned (about every 2 s). Data files are opened or closed automatically for each particle (selected from a video display menu) after a specified number of measurements are taken.

Areas, volumes, weights, etc., of particles are then calculated as desired from suitable relationships. Software developed by us presently allows (a) identification of particle type by taxa (e.g. phytoplankton, fecal pellets, ciliates, zooplankton including copepods with their developmental stages, and stream invertebrates), (b) given established relationships, the option to calculate volumes, dry weight, carbon content, etc., and (c) file management to accumulate totals of variables in desired categories, e.g. total dry weight of copepods.

The Hi-Pad digitizing tablet has a resolution of 0.1 mm. Its working dimensions are 25 × 25 cm; therefore, it can resolve to 1 part in 2500 (±0.04%) in one dimension for an object occupying the entire field. Even this resolution can be increased by adjusting magnifications of the drawing tube and microscope such that the area of the digitizing pad corresponds to less than the complete field of view.

Precision is determined by the size of the object with respect to the field of view. This can be adjusted by simple magnification change. For example, for an object that occupies 25% of the field of view, the precision of measurement is 1 in 600 (±0.25%); for an object that occupies only 4% of the microscope or TV monitor field of view, precision is still 1 in 100 or ±1.0% of actual dimension.

Accuracy within the field of view has been determined by repeated measurements of circular and ellipsoidal particles, which occupied approximately 10% of its width, at different places around the field. Error of measurement around the field was not significantly different from error at the centre of the

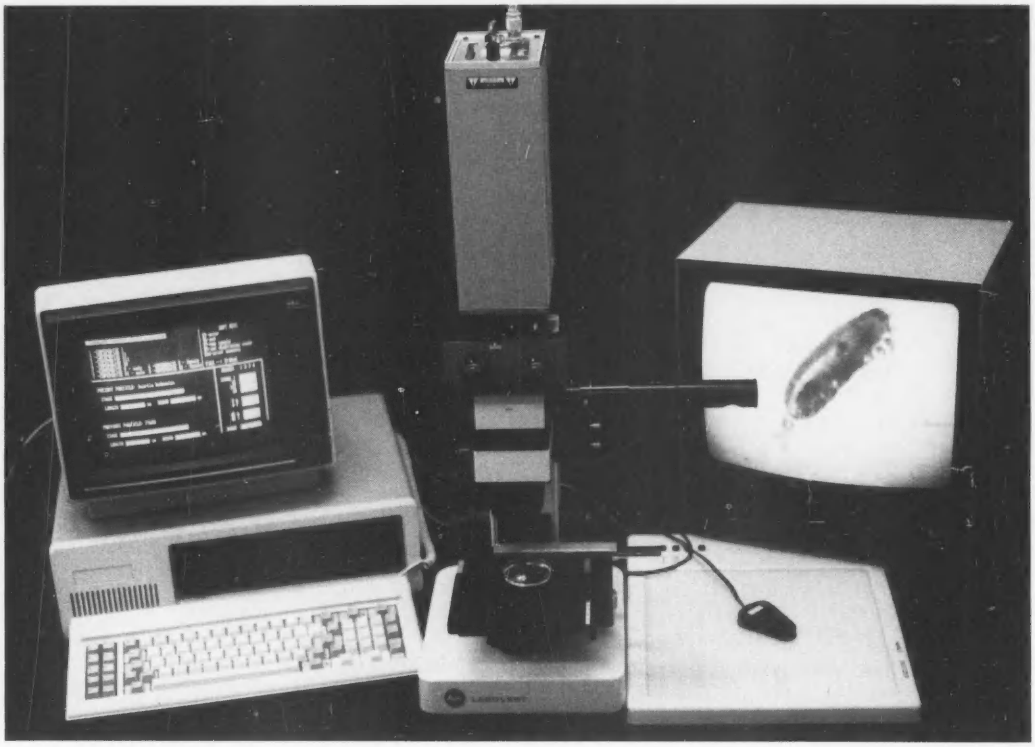


FIG. 1. Measuring system. From left to right: PC with monitor and keyboard; inverted microscope with drawing tube and TV camera; Hi-Pad digitizer with LED cursor, beneath TV monitor.

TABLE 1. Comparison of metasome lengths (μm) of *Centropages furcatus*, measured with the digitizing system and an eyepiece micrometer (n = 10 per stage).

Stage	Measuring system	Mean length	Range of lengths	Standard deviation	95% confidence interval (±)
Adult ♀	Digitizer	1087.6	1085.0–1090.2	3.61	2.58
	Micrometer	1083	1067–1098	22.2	15.9
Adult ♂	Digitizer	1015.3	1011.2–1019.5	5.81	4.16
	Micrometer	996	977–1014	25.9	18.5
Stage V♀	Digitizer	883.4	880.5–886.3	4.04	2.89
	Micrometer	887	862–892	20.7	14.8
Stage IV	Digitizer	593.9	590.8–597.1	4.41	3.15
	Micrometer	615	598–614	23.5	16.8

field; in other words, distortion was not detectable.

Assuming that a researcher or laboratory already possesses a suitable microscope and computer, additional items required for this system are a drawing tube and a digitizing tablet; these will cost about $3000 Cdn. The optional TV camera and monitor will cost about $1500 Cdn.

Applications

Currently we use the system to measure algal cells, ciliates, and fecal pellets in samples of sedimenting material, to measure copepods and tintinnids and derive length–weight relationships, and to determine the biomass of stream invertebrates from published length–weight relationships.

As an example of the precision of this system we present the following data. The metasome length–weight relationship for *Centropages furcatus* generated with this apparatus was ln weight = −22.860 + 3.675 ln length ($R^2 = 0.96$, $p < 0.001$), and the metasome width–weight relationship for the same species was ln weight = −14.709 + 2.949 ln width ($R^2 = 0.96$, $p < 0.001$). Both regressions therefore account for 96% of the variation of weight as a function of linear body dimensions in a natural population of copepods, which includes all copepodite stages and adults.

Separate software packages have been developed by us to sum measurements in appropriate classifications (taxonomic, ecological, or functional) and to convert these to volumes or biomass using appropriate conversion factors, either from the literature or developed in our laboratory (e.g. Middlebrook and

Roff 1986). Output from these BASIC programs is continuously displayed in windowed screen compartments on the monitor, so that user errors can be immediately edited.

Discussion and Comparisons

The "state-of-the-art" in biological measuring is changing rapidly, and systems such as ours tend to evolve independently in several laboratories. We considered publication of this description worthwhile because considerable accuracy and precision can be achieved in routine operation, which allows for very accurate prediction of weights from lengths.

Our system has considerable advantages over conventional measuring using a microscope eyepiece micrometer. Even for an object covering 25% of the screen, maximum precision is often only ±3%. Objects must be manipulated into position and orientation, and flexed or curved specimens are difficult to measure. Errors due to parallax, variation in angles, distance of observer's eye, etc., can be considerable (McCauley 1984). In addition, the operator must estimate length and manually record data — a time-consuming and motion-intensive process subject to errors (see McAllister and Planck 1981).

A comparison of the precision of our measuring system with a conventional microscope eyepiece micrometer, on a random set of specimens, is presented in Table 1. It is evident that the precision of measurement is much lower and that the range, standard deviation, and 95% confidence intervals of measurement are all much greater for the eyepiece micrometer. More importantly, when converted to weights, the errors of length measurements are compounded and lead to errors in weight measurements up to 14% (Table 2). Thus, even when being as precise as possible, eyepiece micrometer measurements compound errors up to at least six times greater than our system. In normal use, the range of error using an eyepiece micrometer will be much larger.

Orientation and position of objects is immaterial when using our measuring system. There is no parallax error because images are combined optically in a field of view. Measurements are made rapidly, and our programs can accept several different measurements on the same object in succession without reorientation. This is advantageous when several measurements such as length, width, oral diameter, etc., are used in combinations to calculate body volume from various geometric shapes (e.g. McCauley 1984). The calculations of volumes are done automatically by our programs once the specified data are entered.

Computerized systems for counting and measuring biological particles can be grouped into three categories. Completely automated nonoptical systems such as the Coulter and Hiac counters cannot distinguish taxonomic groups, and because measurements are approximated to an equivalent spherical diameter, substantial errors in biomass estimates may arise. Several fully automated image analysis systems are commercially available. These can analyze particle size, area, grey level, etc., and measure projected images; most of these systems use a video-overlay technique. Fully automated systems, even when using sophisticated software (e.g. Jeffries et al. 1984; Rolke and Lenz 1984), have poor resolution for biological particles because of the complexities of their shapes, body proportions, and contrast. Image length may therefore be imperfectly defined by the software. Thirdly, semiautomated systems, such as ours (and e.g. Sprules et al. 1981), trade off the speed of commercially available systems against the particle recognition ability of an experienced operator and improved precision and accuracy of measurement.

TABLE 2. Comparison of weights (μg) of *Centropages furcatus* predicted by the equation ln weight = −22.860 + 3.675 ln length, using data from the digitizing system and an eyepiece micrometer.

Stage	Measuring system	Mean weight	Range of weights	Range of weight as % of mean
Adult ♀	Digitizer	17.04	16.85−17.17	1.88
	Micrometer	16.7	15.8−17.7	11.4
Adult ♂	Digitizer	13.22	13.03−13.42	2.94
	Micrometer	12.3	11.5−13.2	13.8
Stage V♀	Digitizer	7.93	7.82−8.08	2.52
	Micrometer	7.7	7.2−8.2	13.0
Stage IV	Digitizer	1.84	1.80−1.88	4.34
	Micrometer	2.1	1.8−2.1	14.2

The major advantages of our system are versatility and precision. It is considerably more accurate than the manual mode of commercially available products using a video-overlay technique. In such instruments an image from a microscope is projected onto a TV monitor, and this is combined with a cross hairs image, generated from a digitizing pad, which is used to target the image. Movement of the digitizing cursor then moves the cross hairs on the monitor. Because of incompatibility between camera, monitor, and digitizer scan lines, rounding errors can considerably reduce precision of measurement. Here, it is important to realize that the accuracy of the system is not that of the digitizing pad, but that of the monitor's resolution. For example, in one commercially available product we found that repeated measurements on an object occupying 10% of the screen differed by more than 5%.

The microscope image in our system is essentially distortion free, and distortion of the TV monitor is of no consequence because microscope and cursor images are combined before projection. Measurements can therefore be reliably made anywhere on the screen even though an image may appear distorted!

Our system is also versatile. Measurements can be made on objects viewed either through the microscope or projected onto a TV monitor, with similar precision. Operator fatigue can be reduced, and consultation between workers is facilitated. Also, because we have used a standard IBM-PC and Hi-Pad digitizer, system components are compatible with future applications such as grey level image analysis.

Acknowledgements

We thank Dieter Wagner and Chris Kay of Wild-Leitz Canada for allowing us to disrupt two of their trade shows, and for assistance in the exploration of alternative designs. We thank L. Chisholm for the data on *Centropages furcatus* produced with this system. This research was supported by the Natural Sciences and Engineering Research Council of Canada.

References

BANSE, K., AND S. MOSHER. 1980. Adult body mass and annual production/biomass relationships of field populations. Ecol. Monogr. 50: 355−379.

JEFFRIES, H. P., M. S. BERMAN, A. D. POULARIKAS, C. KATSINIS, I. MELAS, K. SHERMAN, AND L. BIVINS. 1984. Automated sizing, counting and identification of zooplankton by pattern recognition. Mar. Biol. 78: 329−334.

MCALLISTER, D. E., AND R. J. PLANCK. 1981. Capturing fish measurements and counts with calipers and probe interfaced with a computer or pocket calculator. Can. J. Fish. Aquat. Sci. 38: 466−470.

calculator. Can. J. Fish. Aquat. Sci. 38: 466–470.
McCauley, E. 1984. The estimation of the abundance and biomass of zooplankton in samples, p. 228–265. *In* J. A. Downing and F. H. Rigler [ed.] A manual on methods for the assessment of secondary productivity in fresh waters. Blackwell Scientific Publications, Oxford.
Middlebrook, K., and J. C. Roff. 1986. A comparison of methods for estimating annual productivity of the copepods *Acartia hudsonica* and *Eurytemora herdmani* in Passamoquoddy Bay, New Brunswick. Can. J. Fish. Aquat. Sci. 43: 656–664.
Pearre, S. Jr. 1980. The copepod width–weight relation and its utility in food chain research. Can. J. Zool. 58: 1884–1891.
Peters, R. H. 1984. Methods for the study of feeding, grazing and assimilation by zooplankton, p. 336–412. *In* J. A. Downing and F. H. Rigler [ed.] A manual on methods for the assessment of secondary productivity in fresh waters. Blackwell Scientific Publications, Oxford.
Rolke, M., and J. Lenz. 1984. Size structure analysis of zooplankton samples by means of an automated image analyzing system. J. Plankton Res. 6: 637–645.
Sheldon, R. W., A. Prakash, and W. H. Sutcliffe Jr. 1972. The size distribution of particles in the ocean. Limnol. Oceanogr. 17:2344–2353.
Sprules, W. G., L. B. Holtby, and G. Griggs. 1981. A microcomputer based measuring device for biological research. Can. J. Zool. 59: 1611–1614.

Retardation and Recovery of Growth in Brook Trout Fry (*Salvelinus fontinalis*) Exposed for Various Durations to Acidified Water

W. H. Tam, P. D. Payson, and R. J. J. Roy[1]

Department of Zoology, University of Western Ontario, London, Ont. N6A 5B7

Tam, W. H., P. D. Payson, and R. J. J. Roy. 1986. Retardation and recovery of growth in brook trout fry (*Salvelinus fontinalis*) exposed for various durations to acidified water. Can. J. Fish. Aquat. Sci. 43: 2048–2050.

Brook trout fry (*Salvelinus fontinalis*) were exposed to pH 4.66 for various durations up to 141 d and then returned to neutral water. Growth of test fish was in general significantly lower than that of control fish for exposures up to days 45–78. In four of six groups of acid-treated fish, growth eventually recovered and the growth rates were not different from that of control fish. The results suggested that growth inhibition was induced early in the exposure to sublethally low pH and that recovery in the latter phase of the experiment occurred whether pH remained acidic or was readjusted to neutral.

Des alevins d'omble de fontaine (*Salvelinus fontinalis*) ont été exposés à un pH de 4,66 pendant diverses périodes pouvant atteindre 141 j, avant d'être replacés dans une eau de pH neutre. De façon générale, la croissance des poissons d'essai était significativement inférieure à celle des poissons témoins quand les expositions atteignaient de 45 à 78 j. Chez quatre des six groupes de poissons soumis au milieu acide, il y a eu reprise de la croissance et les taux ne différaient pas alors de ceux des poissons témoins. Les résultats obtenus portent à croire que l'inhibition de la croissance apparaissait au début de l'exposition, à une faible valeur de pH sublétale, et que le rétablissement noté au cours de la dernière étape de l'expérience se produisait que le pH demeure acide ou soit réajusté à une valeur neutre.

Received January 28, 1986
Accepted June 5, 1986
(J8663)

Reçu le 28 janvier 1986
Accepté le 5 juin 1986

Exposure to sublethally low pH often results in reduced growth rate in freshwater fishes both in the field and laboratory (Beamish et al. 1975; Craig and Baksi 1977; Rodgers 1984). The pH levels at which growth inhibition initially occurs have been documented. In brook trout (*Salvelinus fontinalis*), growth was reduced by 23–29% after they were chronically maintained at either pH 5.16 or 4.48 (Tam and Payson 1986). In natural waters, pH can be substantially lower after episodic events such as rainfall (Haines 1981) and snowmelt (Jeffries et al. 1979). Yet, the effect of duration of exposure to sublethally low pH remains unknown. The aim of our investigation was to study the relation between duration of exposure and growth inhibition and to compare the rate of recovery among brook trout either maintained in low pH or returned to neutral pH after a period of exposure to acid.

Table 1. Temperature, pH values, dissolved oxygen, and carbon dioxide concentrations in the water of the fish holding tanks. Results are expressed as mean ± SE (number of observations in parentheses).

Tank	Temperature (°C)	pH	Oxygen (mg/mL)	Carbon dioxide (mg/mL)
A	11.6±0.1(143)	7.36±0.06(14)	8.54±0.28(15)	6.75±0.60(15)
B	11.6±0.1(143)	4.66±0.02(143)	7.58±0.25(15)	31.20±3.61(15)[a]

[a]Measured immediately after the daily addition of sulphuric acid.

Materials and Methods

Two hundred and fifty 4-mo-old brook trout fry were purchased from Goossens Trout Farm (Otterville, Ont.) in April

[1]Present address: Department of Fisheries and Oceans. West Vancouver Laboratory, 4160 Marine Drive, West Vancouver, B.C. V7V 1N6.

TABLE 2. Body weight (g) of brook trout fry maintained at neutral pH and for various durations at pH 4.67. Results are expressed as mean ± SE (number of fish in parentheses).

Group	Duration at pH 4.67 (d)[a]	Days of experiment							
		0	8	14	29	45	78	106	141
1	0	3.7±0.07(168)	4.00±0.12(69)	4.22±0.25(25)	6.34±0.51(13)	8.12±0.28(73)	12.3 ±0.41(59)	15.23±0.54(67)	23.83±0.95(72)
2	141	—	3.02±0.10(53)[b]	3.70±0.13(69)[b]	5.45±0.19(57)	6.46±0.48(18)[b]	11.29±0.48(45)	15.84±1.12(22)	32.27±2.84(20)[b]
3	8	—	—	3.54±0.20(26)[b]	5.11±0.29(13)	7.12±0.41(18)	10.49±0.59(18)	13.24±0.92(19)	21.83±1.68(16)
4	14	—	—	—	5.13±0.50(13)	6.41±0.48(25)[b]	9.44±0.72(19)[b]	13.32±0.81(23)	19.83±1.89(19)[b]
5	29	—	—	—	—	7.01±0.49(23)	10.56±0.80(20)	14.15±1.05(24)	21.96±1.90(20)
6	45	—	—	—	—	—	8.42±0.50(24)[b]	11.71±1.28(10)	17.97±1.81(13)[b]
7	78	—	—	—	—	—	—	13.98±0.94(23)	22.03±2.08(21)

[a]Starting on day 0 of experiment.
[b]Significantly different from group 1 either at the 5 or 1% level (ANOVA and SNK a posteriori comparison of means).

1985. The fish were maintained in a 58 × 208 × 44 cm deep Min-O-Cool fibre glass tank (model MT-700, Frigid Units Inc., Toledo, OH), designated tank A, and were allowed 1 mo to acclimate to laboratory conditions (Table 1). At the end of this period, 170 fish were moved into a similar Min-O-Cool tank (tank B) where with the addition of sulphuric acid the pH was maintained at pH 6.0 for the first 4 d and lowered by 0.5 pH unit every third day until pH 4.5 was reached. A nominal pH 4.5 was maintained in tank B for the duration of the experiment. The experimental period began on the day (day 0) when pH first reached pH 4.5 in tank B. Twenty-eight fish were removed from the acid tank B and returned to the neutral tank A on each of the following days: day 8 (group 3), day 14 (group 4), day 29 (group 5), day 45 (group 6), and day 78 (group 7). The two groups of fish that were kept in the neutral and acid tanks throughout the experiment were respectively called group 1 (control) and group 2. Each group of fish was identified by selectively clipping their fins. The growth rate of the fish was monitored by randomly weighing as many fish as possible (averaging 67% of the fish in each group) on days indicated in Table 2. The quality of laboratory water (alkalinity TIP 78 mg/L), means of dechlorination, and the acidification of water with sulphuric acid have all been reported elsewhere (Tam and Payson 1986). The water in the Min-O-Cool tanks was constantly circulated through activated charcoal filters and refrigeration units. One third of the water was replaced every day. The fish were fed daily to near satiation with commercial trout food. Dissolved oxygen concentration (dissolved oxygen meter, model 57, Yellow Springs Instrument Co., Yellow Springs, OH) and carbon dioxide concentration (carbon dioxide kit, model PCO-S, LaMotte Chemical Products Co., Chestertown, MD) were periodically checked. The experiment was terminated on day 141. Most of the fish mortality occurred when the fry managed to squeeze through the protective screens surrounding the water circulation fans of the refrigeration units.

Results and Discussion

The fish in tank B (group 2) had significantly lower body weight than that of group 1 (control) up to day 45 (Table 2). After that, body weight in group 2 was no longer significantly lowered, but it actually surpassed that of group 1 on day 141. The body weight in groups 3–7 tended to be lower than that of group 1 mainly at or before day 78 of the experiment. The lowered body weight observed in the acid-treated fish could not have been due to handling stress nor to difference in feeding regime, since all acid and control fish were weighed and fed in the same manner. Linear regression analysis was performed on the data in Table 2. The results of the analysis are given in Table 3. The regression lines of groups 1, 3, 6, and 7 are parallel, and the growth rates in groups 2, 3, 6, and 7, as represented by the slopes, were either higher than or the same as that of control fish in group 1.

The results of the experiment suggest that after these brook trout had been exposed to acid, adverse physiological changes, such as electrolyte and chloride ion losses (Leivestad and Muniz 1976), were triggered within the first few days. These stressful changes probably and indirectly caused a transient growth inhibition. Low pH has been shown to result in high blood cortisol, glucose, and protein levels in rainbow trout (*Salmo gairdneri*) (Brown et al. 1984), possibly making these nutrients unavailable for normal growth. In brook trout, after the fish had acclimated to low pH, growth inhibition was fol-

TABLE 3. Linear regression analysis between duration of experiment (X = days) and body weight (Y = grams).

Group	Regression equation[a]	t-test[b]	
		Slope	Y-intercept
1	$0.0062X + 0.53 = \log Y$	—	—
2	$0.0073X + 0.45 = \log Y$	$p < 0.001$	$p < 0.02$
3	$0.0059X + 0.50 + \log Y$	NS	$p < 0.01$
4	$0.0052X + 0.54 = \log Y$	$p < 0.02$	$p < 0.001$
5	$0.0050X + 0.60 = \log Y$	$p < 0.01$	NS
6	$0.0051X + 0.51 = \log Y$	NS	$p < 0.001$
7	$0.0053X + 0.57 = \log Y$	NS	$P < 0.001$

[a]The regression coefficient is significantly different from zero ($p < 0.001$) in all seven groups.

[b]Comparison with group 1; NS = not significantly different from group 1 at the 5% level.

lowed by a recovery phase whether the fish were retained in sublethally low pH or returned to neutral pH. The high carbon dioxide level in tank B probably did not have much direct effect on body weight, as the principal effect of high carbon dioxide was similar to low water pH in inducing acidemia and this effect lasted only a few hours (Eddy 1976; Neville 1979). Our results also imply that once fish have adapted to low pH, subsequent episodic occurrence of low pH may have little effect on the fish. The results also highlight the precautions that must be taken when comparing the results of acute and chronic exposure experiments.

Acknowledgements

This research was supported by a strategic grant (G1569) awarded to W.H.T. by the Natural Sciences and Engineering Research Council of Canada.

References

BEAMISH, R. J., W. L. LOCKHART, J. C. VAN LOON, AND H. H. HARVEY. 1975. Long-term acidification of a lake and resulting effects on fishes. Ambio 4: 98–102.

BROWN, S. B., J. G. EALES, R. E. EVANS, AND T. J. HARA. 1984. Interrenal, thyroid, and carbohydrate responses of rainbow trout (*Salmo gairdneri*) to environmental acidification. Can. J. Fish. Aquat. Sci. 41: 36–45.

CRAIG, G. R., AND W. F. BAKSI. 1977. The effects of depressed pH on flagfish reproduction, growth and survival. Water Res. 11: 621–626.

EDDY, F. B. 1976. Acid–base balance in rainbow trout (*Salmo gairdneri*) subjected to acid stresses. J. Exp. Biol. 64: 159–171.

HAINES, T. A. 1981. Acidic precipitation and its consequences for aquatic ecosystems: a review. Trans. Am. Fish. Soc. 110: 669–707.

JEFFRIES, D., C. COX, AND P. DILLON. 1979. Depression of pH in lakes and streams in central Ontario during snowmelt. J. Fish. Res. Board Can. 36: 640–646.

LEIVESTAD, H., AND I. P. MUNIZ. 1976. Fish kill at low pH in a Norwegian river. Nature (Lond.) 259: 391–392.

NEVILLE, C. M. 1979. Ventilating response of rainbow trout (*Salmo gairdneri*) to increased H ion concentration in blood and water. Comp. Biochem. Physiol. 63A: 373–376.

RODGERS, D. W. 1984. Ambient pH and calcium concentration as modifiers of growth and calcium dynamics of brook trout, *Salvelinus fontinalis*. Can. J. Fish. Aquat. Sci. 41: 1774–1780.

TAM, W. H., AND P. D. PAYSON. 1986. Effects of chronic exposure to sublethal pH on growth, egg production, and ovulation in brook trout, *Salvelinus fontinalis*. Can. J. Fish. Aquat. Sci. 43: 275–280.

LETTERS AND COMMENTS/LETTRES ET COMMENTAIRES

Generalizing Fisheries Models: An Unfortunate Illusion

In a recent paper, Tsoa et al. (1985) address an important shortcoming concerning production functions for fisheries. After noting the primitive form of the Cobb–Douglas production function inherent to the Schaefer model, they proceed to develop a method for estimating the Schaefer model without imposing the usual crude assumption of unitary values for the Cobb–Douglas exponents. Thus, Tsoa et al. attempt to directly estimate the values of these exponents. This represents a potentially significant advance, for as they note, "The generalization results in equilibrium catch and population figures that can be substantially different from conventional Schaefer estimates." The adoption of their model would thus have serious implications, as both steady-state allocations and total allowable catch paths to the steady state may be strongly affected. Thus, the Generalized Model offered by the authors warrants careful examination.

Unfortunately, closer examination reveals a serious flaw. To illuminate this flaw, a brief summary of the Tsoa et al. development of the Generalized Model is helpful.

The two equations of the Schaefer model are

$$(1)\quad Q = kEP$$

and

$$(2)\quad \frac{dP}{dt} = aP - bP^2 - Q$$

where Q, E, P, and t are catch, effort, population, and time, respectively, and k, a, and b are parameters. Most investigators (e.g. for recent examples see Lewis (1982) or Stocker and Hilborn (1981)) estimate the three parameters defined in the Schaefer formulation. Tsoa et al. generalize this model to

$$(3)\quad Q_t = e^k E_t^{\alpha} P_t^{\beta}$$

$$(4)\quad P_t - P_{t-1} = aP_{t-1} - bP_{t-1}^2 - W_{t-1}.$$

Thus, equation (3) is a slightly modified form of the more conventional Cobb–Douglas function:

$$(5)\quad Q = kE^{\alpha}P^{\beta}$$

whereas equation (4) is equation (2) expressed in discrete terms. They then solve this system to produce

$$Q_t = e^k E_t^{\alpha}\left[(1+a)\left(\frac{Q_{t-}}{e^k E_{t-1}^2}\right)^{1/\beta} - b\left(\frac{Q_{t-1}}{e^k E_{t-1}^{\alpha}}\right)^{2/\beta} - W_{t-1}\right]^{\beta}$$

Thus, $Q_t = f(E_t, E_{t-1}, Q_{t-1})$ and the five parameters k, α, β, a, and b may then be estimated by a nonlinear regression technique. Annual time series data on Q_t and W_t are available and utilized by the authors; however, E_t, the effort variable, requires further modelling and proves to be the Achilles Heel of the model. E_t is defined as the total standardized effort expended by all relevant Newfoundland offshore vessels fishing for northern cod in year t. The authors adopt a method whereby they establish relative productivity weights for all gear–vessel combinations. These weights were then determined from a pooled sample of time series and cross-sectional data for the Newfoundland ground fishery for the period 1953–79. The effort variable used is denoted as "dory units" or "equivalent days fishing by handling from a dory." "This variable is then computed as a weighted sum of the days fishing reported for each gear–vessel combination used in the offshore northern cod fleet, the weights being the productivity of that particular gear–vessel combination relative to the productivity of dory handling." Thus, Tsoa et al. have implicitly assumed $\alpha = 1$. At any time t, both the catchability e^k and the population P_t are constant. Thus, by taking the ratio of one day's catch as the relative effort weight, that is,

$$\frac{Q_{ij}}{Q_{nm}} = \frac{E_{ij}}{E_{nm}},$$

the authors have implicitly assumed

$$Q = e^k EP^{\beta}.$$

It is thus not surprising when their estimate for α of 0.975 is close to 1. More importantly, the implicit assumption is inconsistent with the general form of the function, exhibiting a serious flaw in an otherwise elegant approach.

Thus, the authors have not as yet uncovered the philosopher's stone. This is unfortunate, as the results of other studies cited by the authors, Tomkins and Butlin (1975) and Henderson and Tugwell (1979), seriously question the validity of assuming the standard Schaefer model. Furthermore, as Tsoa et al. correctly state, equilibrium catch and population figures can vary substantially depending upon whether their estimates or the original Schaefer assumptions are used. Thus, it is important to reiterate their conclusion that steady-state quota allocations and total allowable catch paths to the steady state may be strongly affected by the production assumptions made. The Generalized Model of Tsoa et al. cannot address this crucial question. However, their paper will remain an important contribution if it elicits the future research necessary to resolve this dilemma concerning production functions. Only then will it be possible to arrive at a truly generalized fisheries model —

Richard Bodell, *Department of Economics, University of Waterloo, Waterloo, Ont. N2L 3G1.* (J8702a)

References

HENDERSON, J. V., AND M. TUGWELL. 1979. Exploitation of the lobster fishery: some empirical results. J. Environ. Econ. Manage. 6: 287–296.

LEWIS, T. R. 1982. Stochastic modelling of ocean resource management. University of Washington Press, Seattle, WA.

STOCKER, M., AND R. HILBORN. 1981. Short-term forecasting in marine fish stocks. Can. J. Fish. Aquat. Sci. 38: 1247–1254.

TOMKINS, J. M., AND J. A. BUTLIN. 1975. Theoretical and empirical approach to fisheries economics. Am. J. Agric. Econ. 26: 105–125.

TSOA, E., W. E. SCHRANK, AND N. ROY. 1985. Generalizing fisheries models: an extension of the Schaefer analysis. Can. J. Fish. Aquat. Sci. 42: 44–50.

Illusion and Reality in Fisheries Models

Professor Bodell's claim is that the method of measuring fishing effort used in Tsoa et al. (1985) is inconsistent with the fisheries production function developed therein, and that this constitutes "a serious flaw to an otherwise elegant approach." This "flaw" is in fact apparent rather than real, however.

We estimated a fisheries production function of the form

$$(1) \quad Q_t = e^k E_t^{\alpha} P_t^{\beta} u_t$$

where Q_t is quantity of fish caught in period t, E_t is fishing effort in period t, P_t is size of fish population in period t, and u_t is an unmeasurable random variable. The variable P_t usually cannot be measured directly, and the main purpose of our paper was to outline techniques with which the influence of P_t can be inferred indirectly from a time series on Q_t and E_t.

In a fishery prosecuted by fleets of different sizes, or using different gear types, fishing effort E_t cannot be measured directly either. While data may exist on days fishing by various vessel-classes, there is no straightforward way of aggregating such data into a measure of fishing effort in the entire fishery. This is possibly why the earliest studies of fish population dynamics concentrated on fisheries which, for regulatory or other reasons, utilized a uniform gear technology, for example, yellowfin tuna (Schaefer 1957) and Pacific halibut (Crutchfield and Zellner 1962).

Professor Bodell implicitly accepts the validity of our technique when applied to a fishery which is exploited exclusively or overwhelmingly with such a uniform fishing technology. Under such conditions, when the same gear is utilized by vessels of similar size and productivity, fishing effort can be reliably measured by a simple aggregate such as total days fishing.

Of course, the northern cod fishery can scarcely be characterized as a single-gear fishery, and so it is necessary to measure the relative impact of effort from several vessel-classes. While we mentioned this only in passing in the paper, we have dealt with this problem extensively elsewhere (Schrank et al. 1980, p. 35–54; Schrank et al. 1984, Chap. 5).

In this reply, we demonstrate that the approach developed in Tsoa et al. (1985) is internally consistent, in that our measurement of fishing effort is compatible with a fisheries production function of the form (1). On this point, Professor Bodell is simply incorrect. The "critical flaw" which Professor Bodell claims exists in our approach is in fact an illusion.

A measure of fishing effort should reflect the impact which a particular fishing technology has on a fish stock. This implies that the measurement of fishing effort should be such that two different vessel-classes, fishing the same stock at the same time, should have the same catch-per-unit-effort. We would like, in other words, to have the relationship

$$(2) \quad \frac{Q_{it}}{E_{it}} = \frac{Q_{jt}}{E_{jt}} \qquad \text{all } i \text{ and } j$$

where Q_{it} and E_{it} are catch and effort by vessel-class i in period t, and similarly for vessel-class j.

In order to construct such a measure, we subjected our data to a decomposition in the form

$$(3) \quad y_{it} = \phi + \gamma_t + \delta_i + v_{it}$$

where y_{it} is the logarithm of catch per day by the vessel-class i during period t. A vessel-class is defined here in terms of both vessel size and gear usage. In this decomposition, ϕ is a benchmark or datum value for y_{it}, γ_t reflects effects specific to period t across all vessel-classes, and δ_i reflects effects specific to vessel-class i across all periods. The variable v_{it} is a random variable reflecting effects unique to the vessel-class i during period t, and so the mathematical expectation of v_{it} is zero ($Ev_{it} = 0$).

Selection of the benchmark value ϕ is clearly arbitrary, since a change in ϕ can be offset by an equal and opposite change in all the γ's or δ's. In our study, we found that there were some vessel-classes with average catch per day not significantly different (statistically) from the sample average, and some years in which average catch per day was not significantly different from the sample average. It seemed natural to select these as the datum. Clearly, however, any datum point would serve equally well.

In the development below, we assume (without loss of generality) a datum point at $i = 1$ and $t = 1$. This implies $\delta_1 \equiv \gamma_1 \equiv 0$, and so manipulating the decomposition (3) and taking its mathematical expectation gives us the relationships

$$\begin{aligned}
\phi &\equiv y_{11} - v_{11} = Ey_{11} \\
\phi + \delta_i &\equiv y_{i1} - v_{i1} = Ey_{i1} \\
\phi + \gamma_t &\equiv y_{1t} - v_{1t} = Ey_{1t} \\
\phi + \delta_i + \gamma_t &\equiv y_{it} - v_{it} = Ey_{it}
\end{aligned}$$

where the condition $Ev_{it} = 0$ is used. Since $y_{it} \equiv \ln (Q_{it}/f_{it})$, where f_{it} is days fishing by vessel-class i during period t, we can write

$$(4) \quad Q_{it} = \exp [\phi + \gamma_t + \delta_i + v_{it}] f_{it}.$$

If we define fishing effort E_{it} as

$$(5) \quad E_{it} = \exp [\delta_i + v_{it}] f_{it},$$

then (4) can be rewritten as

$$Q_{it} = \exp [\phi + \gamma_t] E_{it}$$

and it is evident that (5) meets condition (2). Moreover, if we write total fishing effort $E_t = \Sigma_i E_{it}$, then

$$(6) \quad Q_t = \sum_i Q_{it} = \exp [\phi + \gamma_t] E_t$$

so that it is also true that

$$(7) \quad \frac{Q_{it}}{E_{it}} = \frac{Q_t}{E_t} \qquad \text{all } i.$$

Bodell's point is that equation (6) appears to be incompatible with equation (1) unless $\alpha = 1$. To make (6) compatible with (1), it must be replaced by something like

$$Q_t = \exp [\phi + \gamma_t] E_t^{\alpha}.$$

In this form, the two equations are equivalent when

$$\exp [\phi + \gamma_t] = e^k P_t^{\beta} u_t.$$

Bodell suggests that using the decomposition (3), with its implied assumption of $\alpha = 1$, at best renders incorrect estimates of the δ_i parameters, and may even predispose our estimate of the production function (1) to yield a value of α close to unity. Despite its superficial plausibility, this point is incorrect, as we shall now demonstrate.

Our estimate of fishing effort is derived from the expression (5), with an estimate of δ_i substituted for the expression

$\delta_i + v_{it}$. The estimate was obtained from a least-squares regression equation of observations on y_{it} across a set of vessel-classes $i = 1, \ldots, I$ and a set of periods $t = 1, \ldots, T$. These observations on y_{it} are regressed on two sets of binary dummy variables. One set, denoted by x_{jit}, $j = 2, \ldots, T$, is the set of period-specific dummy variables and is set equal to unity for $j = t$ (the current period for y_{it}) and zero for all other j. The other set, denoted by z_{kit}, $k = 2, \ldots, I$, is the set of vessel-class-specific dummy variables and is set equal to unity for $k = i$ (the current vessel-class) and zero for all other k. The regression equation is thus in the form

$$(8) \quad y_{it} = a + \sum_{j=2}^{T} b_j x_{jit} + \sum_{k=2}^{I} c_k z_{kit} + \epsilon_{it}$$

where ϵ_{it} is a random variable with zero expectation. The coefficients b_j are estimates of the component of y_{it} specific to the jth year, across all vessel-classes, and the c_k are estimates of the component of y_{it} specific to the kth vessel-class, across all periods. It is the set of c_k which is used to estimate the parameters δ_i.

For notational convenience, we shall write (8) in the equivalent form

$$(9) \quad y_{it} = \sum_{1}^{T} b_j x_{jit} + \sum_{2}^{I} c_k z_{kit} + \epsilon_{it}$$

so that $b_1 = a$ and the remaining b_i incorporate the value of a. In matrix notation, this can be expressed in the form

$$\boldsymbol{y} = [\boldsymbol{X}\boldsymbol{Z}] \begin{bmatrix} \boldsymbol{b} \\ \boldsymbol{c} \end{bmatrix} + \boldsymbol{\epsilon}.$$

Here, $\boldsymbol{y}$ and $\boldsymbol{\epsilon}$ are $IT \times 1$ vectors of the y_{it} and ϵ_{it}, arrayed in the order $y_{11}, y_{21}, \ldots, y_{i1}, \ldots, y_{I1}, y_{12}, \ldots, y_{IT}$, $\boldsymbol{b}$ is the $T \times 1$ vector of b_j's, and $\boldsymbol{c}$ is the $(I - 1) \times 1$ vector of the c_j's. The matrix $\boldsymbol{X}$ is the $IT \times T$ array of the x_{jit}, which can be represented in the form

$$\boldsymbol{X} = \begin{bmatrix} \boldsymbol{X}_1 \\ \vdots \\ \boldsymbol{X}_T \end{bmatrix}$$

where $\boldsymbol{X}_i$ is the $I \times T$ matrix with 1's in column i and 0's elsewhere. The matrix $\boldsymbol{Z}$ is the $IT \times (I - 1)$ array of z_{jit}, which can be represented in the form

$$\boldsymbol{Z} = \begin{bmatrix} \tilde{\boldsymbol{V}} \\ \vdots \\ \tilde{\boldsymbol{V}} \end{bmatrix} \text{ where } \tilde{\boldsymbol{V}} = \begin{bmatrix} \boldsymbol{0}' \\ \boldsymbol{I}_{I-1} \end{bmatrix}$$

where $\boldsymbol{0}'$ is a null row vector and $\boldsymbol{I}_{I-1}$ is the $(I - 1)$-order identity matrix.

The least-squares estimator of the vectors $\boldsymbol{b}$ and $\boldsymbol{c}$ is as follows:

$$\begin{bmatrix} \boldsymbol{b}^* \\ \boldsymbol{c}^* \end{bmatrix} = \begin{bmatrix} \boldsymbol{X}'\boldsymbol{X} & \boldsymbol{X}'\boldsymbol{Z} \\ \boldsymbol{Z}'\boldsymbol{X} & \boldsymbol{Z}'\boldsymbol{Z} \end{bmatrix}^{-1} \begin{bmatrix} \boldsymbol{X}'\boldsymbol{y} \\ \boldsymbol{Z}'\boldsymbol{y} \end{bmatrix}.$$

When the formula for inversion of a partitioned matrix (Goldberger 1964, p. 27) is applied, we find that

$$(10) \quad \boldsymbol{c}^* = (\boldsymbol{Z}'\boldsymbol{M}\boldsymbol{Z})^{-1}\boldsymbol{Z}'\boldsymbol{M}\boldsymbol{y}$$

where $\boldsymbol{M}$ is the idempotent matrix

$$\boldsymbol{M} = \boldsymbol{I} - \boldsymbol{X}(\boldsymbol{X}'\boldsymbol{X})^{-1}\boldsymbol{X}'$$

and where it should be noted that

$$\boldsymbol{M}\boldsymbol{X} = \boldsymbol{0}.$$

Let us consider the properties of this estimator. First, from (3)–(5), it is clear that

$$y_{it} \equiv \ln (Q_{it}/f_{it}) = \ln (Q_{it}/E_{it}) + \delta_i + v_{it}.$$

From (7),

$$y_{it} = \ln (Q_t/E_t) + \delta_i + v_{it}$$

which from (1) can be written as

$$(11) \quad y_{it} = k + (\alpha - 1) \ln E_t + \beta \ln P_t + \ln u_t + \delta_i + v_{it}$$

or, in matrix form, as

$$(12) \quad \boldsymbol{y} = \boldsymbol{X}\boldsymbol{\gamma} + \boldsymbol{Z}\boldsymbol{\delta} + \boldsymbol{v}$$

where $\boldsymbol{\delta}$ is the $(I - 1) \times 1$ vector of the δ_i's (recalling that $\delta_1 = 0$), $\boldsymbol{v}$ is the $IT \times 1$ vector of v_{it}'s, and $\boldsymbol{\gamma}$ is a $T \times 1$ vector whose typical element is

$$\gamma_t = k + (\alpha - 1) \ln E_t + \beta \ln P_t + \ln u_t.$$

Substituting (12) into (10), we find that

$$\begin{aligned} \boldsymbol{c}^* &= (\boldsymbol{Z}'\boldsymbol{M}\boldsymbol{Z})^{-1}\boldsymbol{Z}'\boldsymbol{M}[\boldsymbol{X}\boldsymbol{\gamma} + \boldsymbol{Z}\boldsymbol{\delta} + \boldsymbol{v}] \\ &= \boldsymbol{\delta} + (\boldsymbol{Z}'\boldsymbol{M}\boldsymbol{Z})^{-1}\boldsymbol{Z}'\boldsymbol{M}\boldsymbol{v}. \end{aligned}$$

Here, the property $\boldsymbol{M}\boldsymbol{X} = \boldsymbol{0}$ permits the crucial elimination (identically) of the vector $\boldsymbol{\gamma}$ from the expression $\boldsymbol{c}^*$. Taking expectations, we find that

$$E\boldsymbol{c}^* = \boldsymbol{\delta} + (\boldsymbol{Z}'\boldsymbol{M}\boldsymbol{Z})^{-1}\boldsymbol{Z}'\boldsymbol{M}E\boldsymbol{v} = \boldsymbol{\delta},$$

since $E\boldsymbol{v} = 0$, so that the least-squares estimator c_i^* is an unbiased estimator of the relative productivity parameter δ_i. Unfortunately, this does *not* imply that $\exp [c_i^*]$ is an unbiased estimate of $\exp [\delta_i]$ because expectation is a linear operation and is not preserved in exponential transformation. It can be shown (Goldberger 1964, p. 218), however, that

$$(13) \quad E \exp [c_i^*] \approx \exp [\delta_i] \left\{1 + \frac{1}{2} \operatorname{var} c_i^*\right\}$$

is a second-order approximation to the expected value of $\exp [\delta_i]$, and so we can use as an estimate of fishing effort the expression

$$(14) \quad E_t^* = \Sigma E_{it}^* = \Sigma \{\exp [c_i^*]\} f_{it} \Big/ \left\{1 + \frac{1}{2}\sigma_i^2\right\}$$

where σ_i^2 is an unbiased estimate of the variance of c_i^*.

This should be compared with the expression

$$(15) \quad E_t = \Sigma E_{it} = \Sigma \{\exp [\delta_i + v_{it}]\} f_{it}$$

which is derived from equation (5). The expression $\delta_i + v_{it}$ measures the (logarithmic) fishing power of vessel-class i (relative to the datum), and can be decomposed into a systematic component δ_i and an unsystematic or stochastic component v_{it}, the latter having an expected value of zero. The least-squares estimate c_i^* is an unbiased estimator of the systematic component δ_i. The unsystematic component is by nature unobservable.

The expression (14) can be used to measure the *systematic* portion of fishing effort. Under general conditions, we can write

$$\text{plim } E_i^* = \Sigma \exp [\delta_i] f_{it}$$

because the consistency and unbiasedness of the least-squares estimator guarantees that plim $c_i^* = \delta_i$ and plim $\sigma_i = 0$. Thus, E_i^* is under general conditions a consistent estimate of the systematic component of E_t.

We can see then that fishing effort E_t is estimated by E_i^* subject to two types of error: the error in the estimator, which depends on the $c_i^* - \delta_i$, and the error in the equation, which depends on the v_{it}. The former occurs because the relative productivity parameter δ_i is estimated by c_i^* with some error, although the expected value of that error is zero. However, because the least-squares estimator is consistent, this error vanishes as the sample size increases.

The error in the equation results from the use of a constant parameter δ_i to measure the relative productivity of a particular vessel-class over the entire sample, which cannot possibly reflect the unsystematic component v_{it} which is applicable to only one point in the sample. While it is true that this unsystematic component, by definition, has a zero expected value, it can nonetheless be large in particular cases. Moreover, unlike the error in the estimate, this error does *not* vanish as the sample size increases.

In most cases, we would not expect this error to be large. However, we did uncover evidence (Schrank et al. 1984, p. 5.7–5.8) of statistically significant intertemporal interaction effects in some vessel-classes during the years after Extended Fisheries Jurisdiction (EFJ) was declared. In such cases, we altered the c_i^* to incorporate such interaction effects. Analytically, this is equivalent to separating a vessel-class into two distinct and nonoverlapping classes, one active before EFJ and the other active after. This increases the number of vessel-classes, but does not affect the conclusion that the estimate of the c_i^* is unbiased.

Because (14) is an *estimate* of fishing effort, and so fishing effort is generally measured with error, it is no doubt true that there is some error-in-measurement bias in the estimation of the parameters of equation (1). However, this error-in-measurement is a function of the distribution of the v_{it} terms, and is independent of the parameters of the production function (1), including α; in particular, it continues to exist even when $\alpha = 1$. Moreover, in a multitechnique fishery, *any* method of measuring fishing effort is bound to be inexact, and will generate such a bias. The bias does not arise from the assumption of linearity in the relative productivity analysis, as is claimed by Professor Bodell.

Unfortunately, this measurement-error bias does not lend itself to easy analytical examination with our estimator, but analysis of the problem in simpler contexts (e.g. Johnston 1972, p. 281–291) leads us to conjecture that to the extent that it is a problem, it would likely induce a *downward* bias in our estimate of α, not a bias toward unity as suggested by Bodell. However, of all the estimation problems we have encountered in this work, this is the one about which we are least concerned. Successful measurement of fish population is a much tougher problem. Indeed, if population could be measured as easily as fishing effort, fisheries management would be a much simpler task — Noel Roy, Eugene Tsoa, and William E. Schrank, *Department of Economics, Memorial University of Newfoundland, St. John's, Nfld. A1C 5S7.*

(J8702b)

References

CRUTCHFIELD, J., AND A. ZELLNER. 1962. Economic aspects of the Pacific halibut fishery. Fish. Ind. Res. 1(1).

GOLDBERGER, A. S. 1964. Econometric theory. John Wiley & Sons, New York, NY. 399 p.

JOHNSTON, J. 1972. Econometric methods. 2nd ed. McGraw-Hill Book Company, New York, NY. 437 p.

SCHAEFER, M. B. 1957. A study of the dynamics of the fishery for yellowfin tuna in the eastern tropical Pacific Ocean. Bull. Inter-Am. Trop. Tuna Comm. 2: 247–285.

SCHRANK, W. E., E. TSOA, AND N. ROY. 1980. The relative productivity and cost-effectiveness of various fishing techniques in the Newfoundland groundfishery. Discussion Paper No. 180. Economic Council of Canada, Ottawa, Ont. 304 p.

1984. An econometric model of the Newfoundland groundfishery: estimation and simulation. A report submitted to the Department of Fisheries and Oceans. Department of Economics, Memorial University of Newfoundland, St. John's, Nfld. 356 p.

TSOA, E., W. E. SCHRANK, AND N. ROY. 1985. Generalizing fisheries models: an extension of the Schaefer analysis. Can. J. Fish. Aquat. Sci. 42: 44–50.

Multivariate Analysis in Taxonomic Studies: Comment

Field identification of two Atlantic redfishes (*Sebastes mentella* and *S. fasciatus*) appears to be a perplexing problem (Ni 1981; Misra and Ni 1983; McGlade et al. 1983). Kenchington (1986) collected 45 S. *mentalla*, 687 S. *fasciatus*, and 70 unknown *Sebastes* (identified using Kenchington 1986, table 2). He then used principal component analysis (PCA) of morphometric and meristic characters, as well as discriminant function analysis (DFA) or morphometric characters, "to examine the potential value of 15, primarily external, morphological characters as field separators of these species." This comment deals with two questions which occurred to me after reading Kenchington's article: (1) Is principal component analysis appropriate for examining "among-group" variation? (2) Were the criteria Kenchington used for evaluating the DFA valid?

The analyses employed by Kenchington are usually used to describe either the covariation of variables within a given group of observations (PCA), or how groups (defined a priori) are different with respect to a set of variables (DFA) (Pimentel 1979). For redfishes, Kenchington concluded that the two species of interest are too similar to separate using sets of morphometric or meristic measurements.

For his primary evidence of morphological overlap in the redfish species, Kenchington carried out two PCA's using morphometric and meristic characters, respectively. He then constructed bivariate plots of the first three PC scores, identifying each species with different plotting symbols. He claimed that the lack of separation of species groups in his plots was evidence of morphological overlap, stating that "the PCA's clearly show that, in a range of morphometric characteristics, the two species are virtually indistinguishable." This is totally false, as Kenchington's own MANOVA on the same morphological data shows ($F = 56.26$; df = 5,726; $p \ll 0.001$). Clearly, a MANOVA is the appropriate test of how morphologically "distinguishable" the groups are.

PCA describes among-individual variation. Any correspondence between axes which best describe the variation among individual points (as in PCA) and those which describe variation among groups of points (as in DFA) will only occur in particular cases. If there does not happen to be any correspondence (as in Kenchington's data), group similarity cannot be inferred from overlap on PC plots. As an example (Fig. 1), imagine a group of animals for which we have length and width

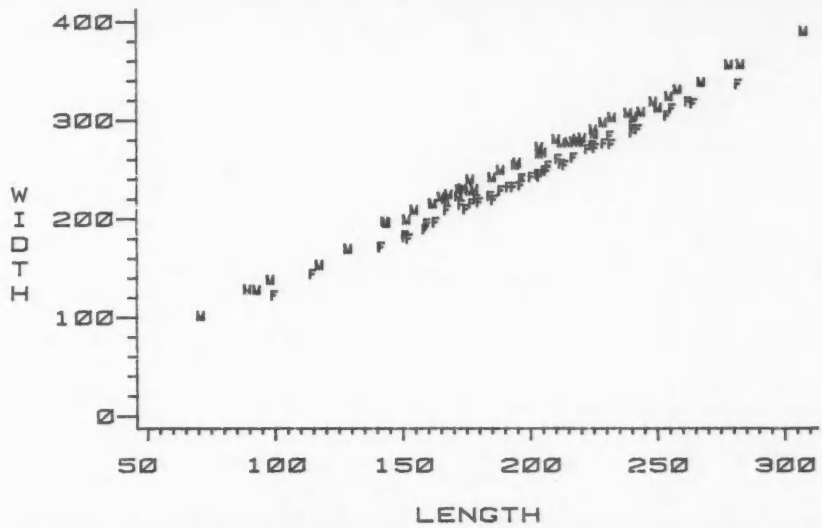


FIG. 1. Simulated data for 50 male (M) and 50 female (F) animals. Standardized PCA showed that less than 1% of the variation in the data is accounted for by the second (and last) principal component. The two sexes differ significantly ($F = 730.16$; df = 1,98; $p < 0.0001$) in their second component scores.

measurements. Both sexes vary tremendously in size, but sexual dimorphism is expressed as a difference in width to length ratio (males are wider at a given length). A histogram of PC1 scores would show total overlap of the sexes. The second, trivial (with respect to individual variation) component would reveal the strong dimorphism present. With more variables, and thus more components, the axis describing among-group variation may be the seventh or eighth principal component, with a small eigenvalue and little associated "variance explained." Thus, overlap of groups on bivariate plots of the first few PC scores is not a valid measure of group differences.

DFA may indeed suggest the nature of the morphological differences between the redfish species. One could make several measurements on an unknown fish and then classify it using a discriminant function calculated from another group of fish of known identity. This appears to be the approach of Ni (1981) and Misra and Ni (1983). They successfully (≈100%) reclassified fish used to compute discriminant functions with meristic (Ni 1981), qualitative morphological (Ni 1981), or morphometric characters (Misra and Ni 1983). Subsequent attempts to classify independently collected fish have not been reported.

Kenchington carried out a DFA on his fish "purely to permit a comparison with Misra and Ni's (1983) results." Reclassification of the 732 fish using five morphometric characters was quite successful (95%), given the extreme imbalance in his design (687 of one species, 45 of the other). It is not true, as Kenchington claims, that his (as well as Ni's (1981) and Misra and Ni's (1983) success in reclassification of the fish is due to "circularity of identification", unless one of the variables in the DFA was also used to do the pre-DFA classification. This may have been true only in Ni's (1981) meristic DFA if he used counts of anal fin rays to classify the fish before the analysis. Kenchington also dismisses the DFA approach because the canonical scores of the two redfish species overlap. Evaluating a DFA by the range of the canonical scores is far too conservative. After performing a DFA, the percentage of misclassified individuals when they are reclassified using morphological data alone seems like a more relevant criterion of success. Jackknife procedures (e.g. Ni 1981) or morphological classification of independently collected and identified (perhaps electrophoretically (McGlade et al. 1983)) fish would be even more useful in assessing the utility of a DFA classification.

The use of external measurements to properly classify Atlantic redfish appears promising (Misra and Ni 1983). Criticism of such an approach because of principal component scores which do not show among-group separation or canonical scores whose ranges overlap is invalid and unproductive — Robert C. Bailey, *Ecology and Evolution Group, Department of Zoology, University of Western Ontario, London, Ont. N6G 3B1.*

(J8780a)

Acknowledgements

R. T. Alisauskas, R. H. Green, L. D. Harder, and K. M. Somers all read the comment and improved it with theirs.

References

KENCHINGTON, T. J. 1986. Morphological comparison of two northwest Atlantic redfishes, *Sebastes fasciatus* and *S. mentella*, and techniques for their identification. Can. J. Fish. Aquat. Sci. 43: 781–787.

MCGLADE, J. M., M. C. ANNAND, AND T. J. KENCHINGTON. 1983. Electrophoretic identification of *Sebastes* and *Helicolenus* in the northwestern Atlantic. Can. J. Fish. Aquat. Sci. 40: 1861–1870.

MISRA, R. K., AND I.-H. NI. 1983. Distinguishing beaked redfishes (deepwater redfish, *Sebastes mentella* and Labrador redfish, *S. fasciatus*) by discriminant analysis (with covariance) and multivariate analysis of covariance. Can. J. Fish. Aquat. Sci. 40: 1507–1511.

NI, I-H. 1981. Numerical classification of sharp-beaked redfishes, *Sebastes mentella* and *S. fasciatus*, from northeastern Grand Bank. Can. J. Fish. Aquat. Sci. 38: 873–879.

PIMENTEL, R. A. 1979. Morphometrics: the multivariate analysis of biological data. Kendall Hunt, Dubuque, IA. 276 p.

Multivariate Analysis in Fisheries Data Acquisition: Reply

I thank Bailey (1986) for his comments on my recent paper (Kenchington 1986). The two questions he poses are indeed matters of concern for future work on the redfishes and similar groups. However, I disagree with his ultimate conclusions, which may have been influenced by his assumption (embodied in his chosen title) that my work dealt with taxonomy, when I was actually concerned with field identification. The specific distinction between *Sebastes fasciatus* and *S. mentella* has been demonstrated, to my satisfaction, by McGlade et al.'s (1983) electrophoretic study.

Beginning with Bailey's first criticism, the "MANOVA" embedded in my discriminant function analysis (DFA) certainly showed a difference between *S. fasciatus* and *S. mentella*, but this is a difference between the locations of the species centroids in morphometric hyperspace. Had the field sampling scheme been suitable and had not Barsukov and Zakharov (1972) in effect demonstrated the same thing 14 yr ago, this might be a useful taxonomic result. However, for the topic of my paper, such a difference in centroids is of very little consequence. In identification, "distinguishability" involves the proportion of individuals lying in some "zone of uncertainty" between the centroids, not proof that the centroids differ.

Bailey then criticises the common approach of using principal components analysis (PCA) to objectively ordinate the data points, preparatory to studying relationships between the individual fish. He objects that subgroups within the data set may be separated on some axis other than one of the major (in terms of eigenvalue) principal components. This is, of course, theoretically correct and it is also possible that subgroup separation lies at an angle to all of the components, both major and minor, where no bivariate component plot will detect it. However, a data set containing subgroups that were entirely nonoverlapping in hyperspace must have some very unusual feature (such as an extreme difference in numbers of individuals in each subgroup) if the axis expressing their separation is to represent only a small proportion of the total variance (and thus to fail to appear as one of the major components).

In practise, for data sets in which the subgroups are clearly distinct, this application of PCA gives valid, positive results. However, Bailey is correct that, when it yields negative results (no apparent separation), this approach is necessarily rather inconclusive. For reasons of clarity and brevity, I did not mention in my paper that (1) although I published the scores on only three principal components, I examined all possible pairs and found broad overlaps of morphometric characteristics between *S. fasciatus* and *S. mentella* throughout and (2) I attempted cluster analyses (which are not subject to this "hidden dimension" problem) of my complete morphometric data set (Kenchington 1983) and was able to separate genera (*Helicolenus* and *Sebastes*) but not the species of *Sebastes*. More convincingly, my DFA (Kenchington 1986), which must have maximised any separation of the two species, showed that the range of morphometric variation of *S. mentella* is entirely contained within that of *S. fasciatus* (insofar as the two are represented in my data set). Thus, my conclusion that (in terms of individual identification) the two species are virtually indistinguishable in morphometry is correct, albeit perhaps insufficiently supported by the PCA results presented in my paper.

Bailey's comments are a valuable reminder that we require a technique which combines the objective, multidimensional presentation of data (as offered by a PCA) with the selection of axes that maximise preselected criteria, such as the separation of subgroups. DFA is not sufficient, since it demands a priori identification of individuals and adherence to rather rigid statistical assumptions that, typically, are not fulfilled by morphological data. PCA followed by some form of axis rotation might be more appropriate. My published data (Kenchington 1983) are available to anyone who might attempt a solution to this problem.

DFA is, as Bailey suggests, an identification technique. The discriminant function should be applied to data from fish of unknown species in order to allocate them to their correct group. The point of disagreement between myself and both Misra and Ni (1983)[1] and Bailey concerns criteria for evaluating the utility of such a function for identification. Certainly the best way, as Bailey suggests, would be to use the function to identify large and representative samples, of known species, that were not included in the initial calculation of the discriminant function. Confidence limits could then be readily derived. The major problem here would be in gathering the test data and, particularly, in identifying the fish, since apart from my recommendation to use electrophoresis (Kenchington 1986), there is no convincing way in which this can be done. (That indeed was my reason for writing.) Some type of Monte Carlo study, using artificial data, might be an alternative. Otherwise, we must resort to some process that, contrary to Bailey's claim, is circular: evaluating the ability of a discriminant function to "identify" the same individuals that were used (with group membership specified a priori) to define the function.

My criterion for evaluating these functions (Kenchington 1986) was the degree of overlap of the two species in canonical variate score. This is probably too crude, but it is conservative and robust.

Misra and Ni (1983), on the other hand, assumed that a low percentage of disagreements between a posteriori identifications and a priori specifications indicates a similarly low error in future identifications of other individuals. Besides the inherent circularity in this process (which must serve to reduce the apparent error), Misra and Ni (1983) may have further underestimated that error for at least two reasons. Firstly, the extreme local variation in morphometry of these fish (Kenchington 1984) and the restricted, nonrandom field sampling scheme that they employed (Ni 1981) will have caused their "species" centroids to differ from the overall centroids for each entire species. Thus, the ranges of morphological characteristics of new samples will tend to diverge from those of the fish used to define the discriminant function. The effects of this factor are not fully predictable, but highly likely to increase the proportion of identification errors. Secondly, Misra and Ni (1983) considered an a posteriori identification to be in error only if its associated probability of being correct was less than 5%. If the onus were reversed, as it would be in practice, and identifications were required that were, say, 95% certain, the proportion of "errors" would rise. In my DFA (Kenchington 1986), for example, they would rise from 4.9 to 31.0% of the sample.

It might be possible to determine a required confidence inter-

[1]While my paper was in press, Misra (1985) withdrew his linear DFA (Misra and Ni 1983) in favour of a quadratic one. Following a correction (Can. J. Fish. Aquat. Sci. 43: 509), this appears to perform slightly better than the earlier analysis (6 rather than 8 "errors" in 198 fish).

val for the specific identifications needed in fisheries data acquisition and a maximum acceptable proportion of unidentifiable specimens. However, this would involve the careful modelling of error propagation throughout the fishery assessment and management process. Until that work is done, Bailey may believe that Misra and Ni's (1983) criterion for evaluating a discriminant function "seems like a more relevant" one than mine, but I suggest that it is seriously misleading.

In conclusion, a multivariate morphometric identification process is far too slow for routine use in the collection of many types of fisheries data (e.g. commercial length–frequencies). For other data types (e.g. age–length data), a process that cannot supply 95%-certain identifications for one third of the fish is hardly likely to be adequate. Thus, my earlier conclusion, that an electrophoretic identification technique is needed for routine use with redfish, remains valid. I therefore suggest that it is Bailey's (1986) criticisms that are "invalid," though perhaps not unproductive — T. J. Kenchington, *CSIRO Division of Fisheries Research, G.P.O. Box 1538, Hobart, Tasmania, 7001, Australia.* (J8780b)

References

BAILEY, R. C. 1986. Multivariate analysis in taxonomic studies: comment. Can. J. Fish. Aquat. Sci. 43: 2054–2055.

BARSUKOV, V. V., AND G. P. ZAKHAROV. 1972. Morphological and biological characteristics of the American redfish. Tr. PINRO 28: 143–173. (Translated from Russian by Fish. Res. Board Can. Transl. Ser., No. 2488)

KENCHINGTON, T. J. 1983. Morphological characters of Scotian Shelf redfish (*Sebastes* spp.) and rosefish (*Helicolenus dactylopterus*). Can. MS Rep. Fish. Aquat. Sci. 1707: 65 p.

1984. Population structures and management of the redfishes (*Sebastes* spp.: Scorpaenidae) of the Scotian Shelf. Ph.D. thesis, Dalhousie University, Halifax, N.S. xviii + 491 p.

1986. Morphological comparison of two northwest Atlantic redfishes, *Sebastes fasciatus* and *S. mentella*, and techniques for their identification. Can. J. Fish. Aquat. Sci. 43: 781–787.

MCGLADE, J. M., M. C. ANNAND, AND T. J. KENCHINGTON. 1983. Electrophoretic identification of *Sebastes* and *Helicolenus* in the northwestern Atlantic. Can. J. Fish. Aquat. Sci. 40: 1861–1870.

MISRA, R. K. 1985. Quadratic discriminant analysis with covariance for stock delineation and population differentiation: a study of beaked redfishes (*Sebastes mentella* and *S. fasciatus*). Can. J. Fish. Aquat. Sci. 42: 1672–1676.

MISRA, R. K., AND I-H. NI. 1983. Distinguishing beaked redfishes (deepwater redfish, *Sebastes mentella* and Labrador redfish, *S. fasciatus*) by discriminant analysis (with covariance) and multivariate analysis of covariance. Can. J. Fish. Aquat. Sci. 40: 1507–1511.

NI, I-H. 1981. Separation of sharp-beaked redfish, *Sebastes fasciatus* and *S. mentella*, from northwestern Grand Bank by morphology of extrinsic gasbladder musculature. J. Northw. Atl. Fish. Sci. 2: 7–12.

ANNOUNCEMENTS/AVIS

C.C.F.F.R. – C.C.R.P.
Vancouver, 5–6 January, 1987

Preliminary Program and Call for Papers

This year's program will begin on Monday, January 5, with the Stevenson lecture, to be presented by Dr. Bill Leggett of McGill University. The Stevenson lecture will be followed by two contributed papers sessions organized around specific themes: (1) Climate and Oceanography and its Effects on Fisheries and (2) Ecology and Population Dynamics of Forage Species. Tuesday, January 6, will begin with an invited papers session: The Biology of Little Known and Unusual Canadian Fishes. This session is being convened by M. C. Healey. The invited papers session will be followed by a poster session, and in the afternoon by a contributed papers session without theme. M. C. Healey is in charge of the overall scientific program, Jake Schweigert is coordinating the contributed papers sessions (all three), and Laura Richards is coordinating the poster session.

This is a ***Call for Abstracts*** for the three contributed papers sessions and the poster session. Type your abstract (maximum 200 words, single spaced). In particular, we want to hear from those of you who want to contribute to one of the sessions having a particular theme. Depending on the number of papers received, we may have to rearrange the sessions. Please forward abstracts for the contributed papers sessions to Jake Schweigert and indicate on your abstract in which session you think the paper belongs. Abstracts for the poster session should be forwarded to Laura Richards. General enquiries should be sent to Mike Healey. Jake, Laura, and Mike are all at the Department of Fisheries and Ocean, Pacific Biological Station, Nanaimo, B.C. V9R 5K6. Deadline for abstracts is November 30, 1986. Earlier submission would, however, be appreciated.

The meetings will be held at the Delta River Inn in Richmond. Dr. G. Geen, Biology Department, Simon Fraser University, is in charge of local arrangements. Contact him for further information about hotel rates, etc.

Conférence canadienne relative à la recherche sur les pêches
Vancouver, les 5 et 6 janvier 1987

Programme préliminaire et appel de documents

Le programme de cette année débutera le lundi 5 janvier avec la conférence Stevenson, qui sera présentée par le Dr Bill Leggett de l'Université McGill. La conférence Stevenson sera suivie de deux séances de présentation de documents organisées autour de thèmes précis : (1) le climat et l'océanographie et ses effets sur les pêches et (2) écologie et dynamique de population d'espèces fourrage. Le mardi 6 janvier débutera avec une séance d'exposés de conférenciers invités : la biologie de poissons du Canada inhabituels et peu connus. Cette séance est convoquée par M. C. Healey et sera suivie par après d'une séance d'affichage et dans l'après-midi d'une séance de présentation de documents sans thème. M. C. Healey est la personne responsable de l'ensemble du programme scientifique, Jake Schweigert coordonne les séances de présentation de documents (les trois), tandis que Laura Richards coordonne la séance d'affichage.

Par la présente, on ***demande des résumés*** pour les trois séances de présentation de documents et pour la séance d'affichage. Veuillez dactylographier votre résumé (maximum de 200 mots à simple interligne). En particulier, nous désirons avoir des nouvelles de ceux d'entre vous qui veulent participer à l'une des séances ayant un thème donné. Selon le nombre de documents reçus, il est possible que nous devions remanier les séances. Veuillez faire parvenir les résumés pour les séances de présentation de documents à Jake Schweigert et indiquer sur votre résumé à quelle séance vous pensez que le document se rattache. Les résumés pour la séance d'affichage doivent être envoyés à Laura Richards. Les demandes de renseignements généraux doivent être adressées à Mike Healey. Jake, Laura et Mike travaillent tous pour le ministère des Pêches et des Océans, à la Station de biologie du Pacifique, Nanaimo (C.-B.) V9R 5K6. La date limite pour les résumés est le 30 novembre 1986. Cependant, nous aimerions les recevoir plus tôt.

Les réunions auront lieu au Delta River Inn, à Richmond. Le Dr G. Geen, département de biologie, Université Simon Fraser, est chargé de faire les arrangements au niveau local. Communiquez avec lui si vous désirez de plus amples informations au sujet des tarifs d'hôtel, etc.

COMING NEXT MONTH/À PARAÎTRE LE MOIS PROCHAIN

International Workshop on Lobster Recruitment

Introduction to the International Workshop on Lobster Recruitment — **A. Campbell**

Session 1: Introduction

Introductory address — **J. E. Stewart**
Dedication — **J. D. Pringle**

Session 2: Lobster Fisheries and Recruitment Variability (Chairman: J. D. Pringle)

Stock definition, recruitment variability, and larval recruitment process in the American lobster, *Homarus americanus*: a review — **G. P. Ennis**
Implications of fluctuations in recruitment in selected crab populations — **G. S. Jamieson**
Problems and perspectives regarding recruitment of spiny lobsters, *Panulirus argus*, to the south Florida fishery — **W. G. Lyons**
Review of the fishery for and biology of the Cape rock lobster *Jasus lalandii* with notes on larval recruitment — **D. E. Pollock**
Recruitment of the puerulus of the spiny lobster, *Panulirus marginatus*, in Hawaii — **C. D. MacDonald**
Prediction of commercial catches of the western rock lobster *Panulirus cygnus* — **B. F. Phillips**
Relationship between indices of juvenile abundance and recruitment in the western rock lobster (*Panulirus cygnus*) fishery — **N. Caputi and R. S. Brown**

Session 3: Larval Distribution Dynamics (Chairman: B. F. Phillips)

California spiny lobster (*Panulirus interruptus*) larval retention and recruitment: a review and synthesis — **J. D. Pringle**
The pelagic phase of spiny lobster development — **B. F. Phillips and P. S. McWilliam**
La répartition horizontale et verticale des larves de homard (*Homarus americanus*) autour des îles de la Madeleine, golfe du Saint-Laurent — **C. Hudon, P. Fradette et P. Legendre**
Swimming ability of larval American lobsters, *Homarus americanus*, in flowing water — **G. P. Ennis**
Application of laboratory studies of larval behavior to fisheries problems — **S. D. Sulkin**

Session 4: Behavior and Movement (Chairman: W. F. Herrnkind)

Relative egg production and abundance of berried lobsters, *Homarus americanus*, in the Bay of Fundy and off southwestern Nova Scotia — **A. Campbell and D. S. Pezzack**
Migratory movements of ovigerous lobsters, *Homarus americanus*, tagged off Grand Manan, eastern Canada — **A. Campbell**
Evidence of migration and homing of lobsters (*Homarus americanus*) on the Scotian Shelf — **D. S. Pezzack and D. R. Duggan**
Recruitment of packhorse rock lobster *Jasus verreauxi* in New Zealand — **J. D. Booth**
Settlement of spiny lobster, *Panulirus argus*, pueruli in south Florida: an evaluation from two perspectives — **J. M. Marx**
Movements of the spiny lobster *Panulirus argus* in south Florida — **D. R. Gregory, Jr. and R. F. Labisky**

Session 5: Recruitment Enhancement
(Chairman: G. Y. Cohan)

Conservation of recruitment of the western rock lobster (*Panulirus cygnus*) by improving survival and growth of undersize lobsters captured and returned by fishermen to the sea
R. S. Brown and N. Caputi

Factors affecting growth and maturation of spiny lobsters, *Panulirus argus*, in the Florida Keys
J. H. Hunt and W. G. Lyons

Session 6: Ecology of Population Structure
(Chairman: J. S. Cobb)

Competition for shelter between lobsters (*Homarus americanus*) and Jonah crabs (*Cancer borealis*): effects of relative size
R. A. Richards and J. S. Cobb

Session 7: Factors Affecting Growth and Reproduction
(Chairman: D. E. Aiken)

Environmental influence on recruitment of the American lobster, *Homarus americanus*: a perspective
D. E. Aiken and S. L. Waddy

Crustacean endocrinology, a review
L. S. Quackenbush

Review of sexual selection and chemical communication in the lobster, *Homarus americanus* **J. Atema**

Multiple fertilization and consecutive spawning in large American lobsters, *Homarus americanus*
S. L. Waddy and D. E. Aiken

Epizootic infestations by nemertean brood parasites on commercially important crustaceans
D. E. Wickham

Influence of light and food on larval American lobsters, *Homarus americanus*
M. D. Eagles, D. E. Aiken, and S. L. Waddy

Nutritional and bioenergetic considerations in the development of the American lobster *Homarus americanus*
G. C. Sasaki, J. M. Capuzzo, and P. Biesiot

Evaluation of the role of nutrition in lobster recruitment
J. D. Castell and J. C. Kean

Session 8: Models of Stock–Recruitment Relationships
(Chairman: M. J. Fogarty)

Modelling stock–recruitment processes in Crustacea: some practical and theoretical perspectives
J. F. Caddy

Effects of environmental forcing on age-structured populations: northern California Dungeness crab (*Cancer magister*) as an example
L. W. Botsford

Effect of assumptions about the stock–recruitment relationship on a lobster (*Homarus gammarus*) stock assessment
R. C. A. Bannister and J. T. Addison

Density-dependent mortality and the relationship between size composition and fishing effort in lobster populations
J. T. Addison

Recruitment dynamics in an American lobster (*Homarus americanus*) population
M. J. Fogarty and J. S. Idoine

Session 9: Implications of the Workshop

Summary of Session 2 — **J. D. Pringle**
Summary of Session 3 — **B. F. Phillips**
Summary of Session 4 — **W. F. Herrnkind**
Summary of Session 5 — **G. Y. Conan**
Summary of Session 6 — **J. S. Cobb**
Summary of Session 7 — **D. E. Aiken**
Summary of Session 8 — **M. J. Fogarty**
Summary of Session 9 — **J. F. Caddy and A. Campbell**
Papers presented at the workshop
Poster papers presented at the workshop
List of participants
Announcements/Avis

Notes

Comparison of continuous and episodic exposure to acidic, aluminum-contaminated waters of brook trout (*Salvelinus fontinalis*) L. K. Siddens, W. K. Seim, L. R. Curtis, and G. A. Chapman **2036–2040**

Variation in major ion concentration of *Cambarus robustus* and *Orconectes rusticus* following exposure to low pH L. Hollett, M. Berrill, and L. Rowe **2040–2044**

High precision microcomputer based measuring system for ecological research J. C. Roff and R. R. Hopcroft **2044–2048**

Retardation and recovery of growth in brook trout fry (*Salvelinus fontinalis*) exposed for various durations to acidified water W. H. Tam, P. D. Payson, and R. J. J. Roy **2048–2050**

Letters and comments / Lettres et commentaires

Generalizing fisheries models: an unfortunate illusion R. Bodell **2051**

Illusion and reality in fisheries models N. Roy, E. Tsoa, and W. E. Schrank **2052–2054**

Multivariate analysis in taxonomic studies: comment R. C. Bailey **2054–2055**

Multivariate analysis in fisheries data acquisition: reply T. J. Kenchington **2056–2057**

Announcements / Avis **2058**

Coming next month / À paraître le mois prochain **2059–2060**

Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

Volume 43, No. 10, October 1986

Volume 43, n° 10, octobre 1986

Articles

Growth and genetic variation of Atlantic salmon (*Salmo salar*) from different sections of the River Alta, North Norway
T. G. Heggberget, R. A. Lund, N. Ryman, and G. Ståhl **1828–1835**

Spatial variability in sedimentary algal microfossils and its bearing on diatom-inferred pH reconstructions
S. S. Dixit and R. D. Evans **1836–1845**

Effect of stream acidification on periphyton composition, chlorophyll, and productivity
P. J. Mulholland, J. W. Elwood, A. V. Palumbo, and R. J. Stevenson **1846–1858**

Metabolic changes during the reproductive migration of two sympatric coregonines, *Coregonus artedii* and *Coregonus clupeaformis*
H. Guderley, P. Blier, and L. Richard **1859–1865**

Characterization of cloned mitochondrial DNA from the teleost *Fundulus heteroclitus* and its usefulness as an interspecies hybridization probe
L. I. González-Villaseñor, A. M. Burkhoff, V. Corces, and D. A. Powers **1866–1872**

Methyl mercury levels in a polluted prairie river–lake system: seasonal and site-specific variations, and the dominant influence of trophic conditions
T. A. Jackson **1873–1887**

Limestone slurry reduces physiological stress and increases survival of Atlantic salmon (*Salmo salar*) in an acidic Norwegian river
B. O. Rosseland, O. K. Skogheim, H. Abrahamsen, and D. Matzow **1888–1893**

A new view of the predation cycle of a planktivorous fish, white crappie (*Pomoxis annularis*)
W. J. O'Brien, B. I. Evans, and G. L. Howick **1894–1899**

Patterns in biomass and cover of aquatic macrophytes in lakes
C. M. Duarte, J. Kalff, and R. H. Peters **1900–1908**

Environmental factors regulating the seaward migration of European silver eels (*Anguilla anguilla*)
L. A. Vøllestad, B. Jonsson, N. A. Hvidsten, T. F. Næsje, Ø. Haraldstad, and J. Ruud-Hansen **1909–1916**

Impact of atrazine on periphyton in freshwater enclosures and some ecological consequences
D. Herman, N. K. Kaushik, and K. R. Solomon **1917–1925**

Biomass and production of fishes in natural and channelized streams
C. B. Portt, E. K. Balon, and D. L. G. Noakes **1926–1934**

Experimental analysis of the direct and indirect effects of an omnivorous filter-feeding clupeid on plankton community structure
R. W. Drenner, S. T. Threlkeld, and M. D. McCracken **1935–1945**

Selection for adult size in female coho salmon (*Oncorhynchus kisutch*)
L. B. Holtby and M. C. Healey **1946–1959**

Photosynthetic characteristics of lotic periphyton
S. Jasper and M. L. Bothwell **1960–1969**

Winter habitat utilization by stream resident brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*)
R. A. Cunjak and G. Power **1970–1981**

Population estimates of white whale, *Delphinapterus leucas*, in James Bay, eastern Hudson Bay, and Ungava Bay
T. G. Smith and M. O. Hammill **1982–1987**

Habitat utilization and behavioral interaction of juvenile Atlantic salmon (*Salmo salar*) and rainbow trout (*S. gairdneri*) in tributaries of the White River of Vermont
W. E. Hearn and B. E. Kynard **1988–1998**

Effects of flooding on dissolved and suspended nutrients in small diked marshes
J. A. Kadlec **1999–2008**

Input–output nutrient budgets for small diked marshes
J. A. Kadlec **2009–2016**

Investigation sur la toxicodynamique du cyanure et sur sa biotransformation chez la truite arc-en-ciel (*Salmo gairdneri*)
P. Raymond, G. Leduc et J. A. Kornblatt **2017–2024**

Estimating the abundance of stone-dwelling organisms: a new method
F. J. Wrona, P. Calow, I. Ford, D. J. Baird, and L. Maltby **2025–2035**

(*continued inside back*)

©Minister of Supply and Services Canada 1986
Ministre des Approvisionnements et Services Canada 1986

CJFSDX43(10) 1827–2060 (1986)
ISSN 0706-652X

Subscription rates / Abonnements

Canada: Institutional / Collectif $150; Personal / Personnel $50
Other countries / Autres pays: Institutional / Collectif $180; Personal / Personnel $60
Single issue / Par numéro: $11.95 (Canada); $14.35 (Other countries / Autres pays)